Testing Western North American Fir Species for Genetic Tolerance and Resistance to Balsam Woolly Adelgid

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Abstract

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Balsam woolly adelgid (BWA), *Adelges piceae* (Ratzeburg) (Hemiptera: Adelgidae), is an invasive insect in North America that is native to Europe. Throughout its introduced range in North America, it affects true fir (*Abies* sp.) species via canopy and stem infestations. In this study, I tested 7 different fir species ranging in their presumed level of susceptibility and tolerance to BWA by inoculating seedlings using two approaches: a new modified passive inoculation and manual inoculation. I also aimed to reconstruct the BWA infestation history on the Gifford Pinchot National Forest, Washington, using dendrochronology. The new modified passive method was successful in inoculating seedlings; for example, 141 of 159 seedlings were successfully inoculated using this approach, which suggests this can be used in future efforts that seek to test true fir species in their resistance and tolerance to BWA. All North American species were highly susceptible to BWA with the Eastern species having higher BWA densities, regardless of seedling size. Both the modified passive and manual inoculation methods revealed that two European species, Nordmann fir (*Abies nordmanniana*) and Turkish fir (*Abies bornmuelleriana*), which are the evolutionarily-native hosts of BWA, developed fewer number of gouts and produced less new growth in response to BWA feeding than the Western (grand fir, *Abies grandis*, and subalpine fir, *Abies lasiocarpa*) and Eastern (Balsam fir, *Abies balsamea*; Fraser fir, *Abies fraseri*; and Canaan fir, *Abies balsamea* var. *phanerolepis*) North American species tested. When using dendrochronological approaches on tree cores collected from the Gifford Pinchot National Forest, the residuals from a tree growth model showed an increase in tree ring widths in the 1950s, which is a previously reported tree growth response to BWA feeding and one that coincides with prior reports of the initial establishment of BWA in this National Forest. The results of this research reinforces the known range of susceptibility of the 7 fir species and furthers our knowledge about BWA in the PNW.

TABLE OF CONTENTS

List of Tables

List of Figures

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Dedication

This thesis is dedicated to Elizabeth Willhite whose knowledge and experience of the parent trees on the Gifford Pinchot National Forest was instrumental to my thesis.

Chapter 1. An introduction to research on Balsam woolly adelgid (*Adelges piceae*) in the Pacific Northwest

Balsam woolly adelgid (BWA), *Adelges piceae* (Ratzeburg) (Hemiptera: Adelgidae), is an invasive insect in North America that is native to Europe. It was first detected in the early 1900s in Eastern North America and is suspected it was introduced via infested nursery stock (Balch 1952, Ragenovich and Mitchell 2006). In North America, it affects true fir (*Abies*) species found throughout the continent. Genetic analyses have suggested that there have been at least two different introduction events, one in Eastern North America and one in Western North America (Havill et. al. 2021). Balsam woolly adelgid was first detected in Brunswick, Maine in the early 1900s (Kotinsky 1916) and subsequently detected in the Willamette Valley, Oregon, in the early 1930s, where it was infesting grand fir (*Abies grandis*); however, it was not considered a serious pest until 1954 when it was found causing high mortality in stands of Pacific silver fir (*Abies amabilis*) around Mount St. Helens (Johnson and Wright 1957, Mitchell 1966). Since its first detection in the Pacific Northwest, BWA has spread throughout the Cascade Mountain Range in Oregon and Washington as well as coastal areas and lowlands (Mitchell 1966). At present, BWA is established in the following U.S. states: Alaska, California, Connecticut, Idaho, Maine, Maryland, Massachusetts, Michigan, Montana, New Hampshire, New York, North Carolina, Oregon, Pennsylvania, Tennessee, Utah, Vermont, Virginia, Washington, Wisconsin, and West Virginia (EDDMapS 2025).

Within Washington, BWA has been established in the Gifford Pinchot National Forest (GPNF) since the 1950s. Recently in 2024, ~12,261 hectares in Washington were killed by BWA, (Washington Department of Natural Resources 2025), including areas of the GPNF. However, despite this and the ~70 year presence of BWA in the GPNF, observations of relatively healthy, individual true fir trees have been reported, even in stands close to areas of high true fir mortality (Fig. 1) (E. Willhite, USDA Forest

Service, unpublished data). The persistence of these surviving host trees could suggest that there is a mechanism of resistance or tolerance to BWA, either genetically, environmentally, or some combination of both. Therefore, the USDA Forest Service began collecting seeds from these surviving true firs, from the 1970s to the 1990s, as a means to study the potential of genetic and environmental factors playing a role in their persistence. Seedlings from these sourced seeds constitute a primary focus of this thesis.

Infestation of true firs by BWA can be detected by examining the canopy and the bole of a host tree. Canopy infestations can be characterized by apical meristem dieback, needle loss, and the presence of gouting, which is swelling at the nodes and tips of branches as a response to BWA feeding (Ragenovich and Mitchell 2006). A bole infestation can be determined by the presence of life stages, such as the “woolly” ovisacs deposited by females, which can be detrimental to a tree under high densities due to disruptions in the hydroconductivity of the host tree (Balch 1952, Greenbank 1970, Chadbourn 2020). Rotholz, “red wood” in German, which is characterized by a red ring similar to compression wood (Fig. 2), may also be present in a tree’s annual rings. It has been noted that summerwood increases by 75 to 80 percent in rings affected by BWA infestation (Doerksen and Mitchell 1965). Annual rings affected by BWA are larger in size leading to increased radial growth, but will decrease after some time (Hain 1988, Leif 2019). The use of dendrochronological methods on the surviving parent host trees in the GPNF to reconstruct the BWA invasion history in this area constitutes a secondary objective of this thesis. For the primary objective of my thesis, I tested seven different fir species for their susceptibility to BWA. Fir seedlings included two species native to Europe, three species native to Eastern North America, and two species native to the Pacific Northwest. The seeds of the two fir seedlings from the Pacific Northwest included those sourced from 30 individual grand fir (2) and subapline fir (*Abies lasiocarpa*) (28) trees located on the GPNF by the USDA Forest Service. Seedlings were inoculated using two different approaches: a modified passive method, based on Newton et. al. (2011), and a manual method, and analyzed for successful BWA establishment, the presence of the F1 generation, and the effects of BWA on the host tree gouting response and growth.

In the second objective of my thesis, I reconstructed the infestation history of BWA in the GPNF using dendrochronology. A total of 17 individual fir trees were visited in the summer of 2024, of which 13 were trees from which seeds were sourced and used in the first objective. Plot data was also taken from these sites along with soil probes to measure environmental factors that may be contributing to resistance or tolerance to BWA. Although both objectives were designed as separate experiments, they also complement each other in the overall objective of the thesis as the Pacific Northwest seedlings used in Objective 1 were sourced from the trees cored in Objective 2. Therefore, both objectives are presented in Chapter 2.

Chapter 2. Susceptibility of evolutionarily native and naïve true fir species to Balsam woolly adelgid (*Adelges piceae*)

Introduction

Balsam woolly adelgid (BWA), *Adelges piceae* (Ratzeburg) (Hemiptera: Adelgidae), is an invasive insect in North America that causes mortality in native true fir (*Abies spp.*). It is native to Europe where it feeds on European silver fir (*Abies alba*) (Greenbank 1970). It was first reported in North America in 1916 (Kotinsky 1916), who identified the species from bark samples of balsam fir (*Abies balsamea*) collected from New Hampshire. Samples collected from Maine in 1908 were also infested with BWA (Kotinksy 1916). It is suspected that the species was introduced from Europe on infested nursery stock (Balch 1952, Ragenovich and Mitchell 2006). Subsequent new detections were reported in Nova Scotia, Prince Edward Island, and Vermont (Balch 1952). However, based on infestation age and its abundance on North American true firs, it is suspected that BWA was well established in Maine and Nova Scotia before 1908 and 1910, respectively (Balch 1952).

In 1928, BWA was first detected on the West Coast of the United States in San Francisco, California, where it was infesting grand fir (*Abies grandis*), noble fir (*Abies procera*), and its native host European silver fir (Annand 1928). By 1930, BWA was found infesting grand fir in the Willamette Valley, Oregon, but it was not considered a serious pest until 1954 when it was found causing high mortality in stands of Pacific silver fir (*Abies lasiocarpa*) around Mount St. Helens (Johnson and Wright 1957, Mitchell 1966). In 1958, BWA was detected on Pacific silver fir, planted as an ornamental tree, in British Columbia; subsequent efforts to delimit its infested range revealed that it was present on native and ornamental firs near Vancouver and southern Vancouver Island (Zilahi-Balogh et. al. 2016).

Balsam woolly adelgid has continued to spread in Eastern and Western North America since its establishment. In the United States, it has been reported in California, Idaho, Maryland, Maine, Montana, New Hampshire, New York, North Carolina, Oregon, Pennsylvania, Utah, Vermont, Washington, West Virginia, and Wyoming (Fei 2024). Recent molecular analyses have shown that there are three subspecies of *Adelges* in North America: *Adelges piceae piceae* (Ratzeburg), *Adelges piceae canadensis* (Merker and Eichhorn) and *Adelges piceae occidentalis* (Foottit and Mackauer) (Havill et. al. 2021). On the west coast of North America, *A. piceae piceae* and *A. piceae occidentalis* are present though the latter has only been reported in British Columbia (Havill et. al. 2021).

Life history of Adelgidae with attention to BWA

Globally, there are ~50 described species within Adelgidae, which is in the superfamily Phylloxeroidea, which is phylogenetically related to Aphidoidea. They have a unique life cycle where they generally alternate between two host species throughout the different generations, which is known as a holocyclic life cycle. For all species within Adelgidae, the primary host is a species of spruce (*Picea* spp.) while the secondary host is a species within *Abies*, *Larix*, *Pseudotsuga*, *Tsuga*, or *Pinus* (Havill and Foottit 2007). It can take up to two years and five generations for a species to complete one holocyclic life cycle. Species of Adelgidae reproduce sexually on the primary host where galls are produced, and asexually on the secondary host (Havill and Foottit 2007).

Populations of Adelgidae species can also be considered as being anholocyclic where they only reproduce on the secondary host (Havill and Foottit 2007). For example, in its native habitat in Europe, BWA is holocyclic where it undergoes asexual reproduction on *A. alba*, and sexual reproduction on Caucasian spruce, *Picea orientalis* (Havill et. al. 2021). In its introduced range in North America, *P. orientalis* is largely absent on the natural landscape and outside of botanic gardens and arboreta. Thus, in North America, BWA is considered anholocyclic, reproducing asexually on *Abies* species. Sexuparae of BWA, which are adults that sexually reproduce and must do so on *P. orientalis*, are likely produced in North America but are considered as non-viable due to the lack of its primary host (Havill and Foottit 2007).

Balsam woolly adelgid females will oviposit once, producing 30-100 eggs (Mitchell 1960). After hatching, crawlers, which are the only mobile stage, move to a new site to feed. Crawlers may also be transported longer distances by wind (Ragenovich and Mitchell 2006). Transport through phoresy, such as on wildlife, is thought to be inconsequential (Woods and Atkins 1967). Once a crawler has selected a suitable feeding site, such as a branch, twig, or bole of the tree, it will then insert its long stylet through the bark where it feeds on the sap (Mitchell 1966, Ragenovich and Mitchell 2006). This first instar is known as a neosisten and is the only stage able to survive winter temperatures (Ragenovich and Mitchell 2006). It develops through two more instars before becoming an adult female, after which it produces a wool-like material and oviposits within it (Balch 1952). The number of generations produced within a year varies depending on elevation and temperature. In the Pacific Northwest, at low elevations, four generations have been observed while at higher elevations, only two have been reported (Mitchell et. al. 1961).

The act of BWA feeding from the tree causes damage to the host. Depending on the infestation site, the tree will respond differently. For example, if BWA nymphs are feeding on the twigs and smaller branches of a tree, a gouting response will occur near the infestation site (Mitchell 1966). If feeding on the bole, a compression-like growth ring will occur, which is referred to as rotholz (Mitchell 1966; Leif 2019). Stem infestations can also lead to water stress due to structural alterations of xylem from BWA feeding (Chadbourn 2020). With time, needles on the inner branches will begin to yellow, the tree will lose dominance in the apical meristem and tree growth will become stunted or severely reduced (Rideout et. al. 2024; Ragenovich and Mitchell 2006). Infested trees will typically die within 2-10 years, but heavily infested trees can die within 2-3 years (Rideout et. al. 2024).

Balsam woolly adelgid in the Pacific Northwest

When BWA was first detected in the Pacific Northwest in the early 1950s, it was found as north as Mt. Rainer in the Cascades, throughout the Toutle, Lewis and Green River drainages near Mt. St. Helens and as south as Mt. Hood in Oregon with spot infestations nearing Crater Lake National Park (Johnson and Wright 1957). It was also detected along Washington's Pacific coast in the Pacific County and Kelso-Longview area and Oregon’s coast in Polk and Lincoln counties (Johnson and Wright 1957). Aerial detection of tree mortality in the Pacific Northwest attributed approximately 111,693 ha in 1954; 119,382 ha in 1955; and 144,068 ha in 1956 due to BWA. In 1956, aerial detection surveys reported more than 80,000 ha of BWA-caused mortality in Washington (Johnson and Wright 1957). Aerial detection surveys continue to report BWA damage with the most recent in 2025 reporting ~12,261 ha of BWA-caused damage, similar to the recent 10-year average of ~12,909 ha per year (Washington State Department of Natural Resources 2025).

Shortly after the discovery of BWA in the cascades, the Pacific Northwest Forest and Range Experiment Station and Weyerhaeuser Company began investigations on BWA (Mitchell and Buffman 2001). These studies included examining potential biological controls, long-term monitoring plots along the Cascades, and the effects of BWA on wood anatomy (Mitchell and Wright 1967; Mitchell and Buffman 2001; Doerksen and Mitchell 1965). In addition, there have been some prior attempts to use dendrochronological methods to decipher BWA infestation history. Dendrochronology is the study of the variability of tree ring widths to determine an environmental change or to date an event. Tree rings can record events such as weather variability, fires, hurricanes, and insect outbreaks (Kipfmueller and Swetnam 2005). In the case of BWA, infestations can be dated with the presence of rotholz and traumatic resin ducts and in the absence of rotholz, increased radial growth can be used (Doerksen and Mitchell 1965; Leif 2019). It was a successful method in Eastern North America where BWA infestations were dated earlier than when observations were made, and where the author observed that exceptionally cold winters resulted in less severe BWA infestations (Leif 2019).

Materials and Methods

Objective 1: Testing for resistance or tolerance to balsam woolly adelgid

The first objective was to determine if Pacific Northwest native firs have a genetic resistance or tolerance to BWA, and to test if a modified method for inoculating seedlings with BWA is effective when testing for resistance or tolerance. The USDA Forest Service, Forest Health Protection, reported that in the 1970s some true fir stands on the GPNF have seemingly withstood BWA pressure while other infested stands have been largely killed. This motivates questions regarding the resistance or tolerance of these stands to BWA, and/or if environmental factors, such as habitat quality, play a role in enhanced resistance or tolerance to BWA infestation. Seeds from five grand fir (three of which are from the GPNF) and twenty-eight subalpine fir (*Abies lasiocarpa*) were collected from 1978 to 1995 and stored in the USDA Forest Service Dorena Genetic Resource Center, Cottage Grove, OR. In 2020 and 2021, the seeds were sowed by the USDA Forest Service and transported to the Washington State University Puyallup Research and Extension Center. Seedlings that were used in this project were from five grand fir families (i.e., seeded from the same parent tree) and nine subalpine fir families, which are native to Western North America. Also included in the study were three fir species native to Eastern North America and two fir species native to Europe. Grand and subalpine fir are known to be susceptible to BWA infestation. Balsam fir (*Abies balsamea*), Fraser fir (*Abies fraseri*), and Canaan fir (*Abies balsamea* var. *phanerolepis*) are native to Eastern North America and have been reported to be susceptible to BWA (Balch 1952, Amman and Speers 1965). Nordmann fir (*Abies nordmanniana*) and Turkish fir (*Abies bornmuelleriana*) are native to Europe and co-evolved with BWA and thus tend to be resistant and/or tolerant to BWA infestation; including both native host species provided a basis for quantifying resistance or tolerance in evolutionary-naive host species. The number of seedlings per species and species families used in this study is presented in Appendix 1.

Inoculation Approaches

To test for resistance or tolerance to BWA, seedlings were inoculated with BWA using one of two methods: a modified passive approach and manual approach. The modified passive approach was based upon Newton et al. (2011) in which the authors suspended BWA-infested bolts over seedlings to passively inoculate them with BWA for subsequent testing for resistance or tolerance. In this approach, it is assumed that BWA crawlers, which are extremely small (~0.4 mm; Balch 1952), drop from the infested bolts onto the seedlings, which allows for a relatively easy method to infest seedlings for experimental studies. However, this approach requires the cutting and suspending of infested bolts.

Modified Passive Inoculation Approach

In the modified passive approach used in this study, I explored the use of an existing block of infested fir trees to determine if crawlers would drop onto seedlings, which eliminates the need to cut and suspend infested bolts. To accomplish this, I placed 280 seedlings directly under a BWA-infested block of balsam fir located at the Washington State University Puyallup Research and Extension Center (Fig. 3). The approach required little set-up and maintenance, while still mimicking the BWA infestation process under natural conditions. Seedlings were placed under the BWA-infested block on 15-April-2024. The timing of this experiment was chosen to be close to the time when females oviposit but before crawlers hatch (Mitchell et al. 1961; Rideout et al. 2024). From the available seedlings provided by the USDA Forest Service (~1,500), a total of 40 grand fir and 40 subalpine fir seedlings were randomly selected from all five grand fir and all nine subalpine fir families. Additionally, 40 randomly selected seedlings from each of the Nordmann, Turkish, Fraser, Canaan, and balsam fir families were also used, for a total of 280 seedlings. Due to space limitations under an infested tree in the block, seedlings were placed in clusters such that each cluster had equal representation of species. This experiment was only conducted in 2024 due to the success in infesting seedlings, thereby indicating the utility of this approach even with only one year of data, and to allow for two years of data to accomplish the more labor- and time-intensive manual inoculation approach.

Manual Inoculation Approach

In the manual infestation approach, individual infested twigs from the BWA-infested balsam fir block, and from different areas where seedlings from the passive experiment were located, collected and manually attached to each individual seedling using twist ties (Fig. 4). In 2024 and 2025, twig collection occurred in mid-April and early May before crawlers emerged. In 2024, a total of 20 seedlings were randomly selected from each of the Nordmann fir, Turkish fir, Fraser fir, Canaan fir, and balsam fir. From the five grand fir families, 10 seedlings were randomly selected from each family, for a total of 50. From the seven subalpine fir families, 10 seedlings were randomly selected, for a total of 70; two subalpine fir families were not included for this method due to insufficient replicates. From each species, half of the seedlings were randomly selected to receive a twig with only one female, and the other half were inoculated with a density of females ranging from four to thirteen. Using different initial densities of BWA allowed me to measure establishment success given variation in host species susceptibility. In 2025, inoculations were based on the availability of remaining seedlings and the data gaps from 2024. A total of 113 seedlings were used in 2025; grand, Canaan, and Fraser fir each had 20 seedlings; subalpine fir had 21 seedlings, balsam fir 16 seedlings, and Turkish fir 15 seedlings. The Washington State University Puyallup Research and Extension Center provided 10 of the 15 Turkish fir seedlings in 2025 to attain replication goals. Due to insufficient remaining seedlings for Nordmann fir, the species was not included in 2025. Seedlings in 2025 were inoculated with either 4, 6, 7, 8, 9, 10, or 11 females depending on data gaps in replication efforts (Fig. 5).

Processing of infested seedlings

For both inoculation approaches, we allowed for BWA to complete at least one generation on inoculated seedlings, which was generally completed by late summer-to-early fall (Fig. 6); degree days were calculated using weather data from Washington State University's AgWeatherNet Puyallup station (AgWeatherNet). At that time, seedlings were harvested from containers at the Washington State University Puyallup Research and Extension Center, placed into ziploc bags, and transported to the University of Washington. Seedlings were either stored under ambient conditions, or placed in cold storage (-20 C) until processed.

To measure the effect of BWA on seedling growth, I measured the length of new growth (Fig. 7) and root collar diameter, the latter of which was used as a covariate to better isolate the direct effect of BWA feeding on seedling growth. Seedlings were also examined for the presence of gouting; all gouts were counted without any time restrictions. These measurements were taken on all seedlings used in both the modified passive and manual inoculation approaches.

I also microscopically examined seedlings for the presence of BWA life stages. I recorded both ovisacs produced from the initial completed generation and subsequent nymphal stages which are < 1mm in size (Fig. 8); these are collectively referred to as the F1 generation. I used both untimed and timed examinations but because untimed inspections were time-consuming, only a subset of seedlings manually inoculated from 2024 were analyzed in this manner. In untimed examinations, I randomly selected 100 seedlings across all species to count BWA individuals comprising the F1 generation. In this approach, seedlings were inspected without a time limit. In seedlings inoculated with one female, I inspected the closest whorl to the inoculation site such that all the branches included in the whorl were microscopically inspected along with 5cm of the stem above and below the inoculation site. In seedlings inoculated with more than one female, I microscopically inspected the whorl that was centered between inoculation sites, and an additional 5cm of the stem on each side of the whorl.

Timed examinations were used in seedlings inoculated with the modified passive approach in 2024 (n=280), seedlings manually inoculated in 2024 that were not selected for the untimed examinations (n=43), and seedlings manually inoculated in 2025 (n=113). In this approach, I used a 5-minute scan focusing on the buds of the new growth as the untimed examinations suggested that BWA was mainly found on the buds as opposed to the branches and stem. Once seedlings were examined, they were placed in the freezer (-20 C) for at least two days to kill BWA prior to disposal.

Hypotheses

I hypothesized that the North American fir species would be more susceptible to BWA than the European host species as the former are considered evolutionarily-naïve and thus have not developed adequate defenses against BWA feeding. Specifically, I hypothesized that I would find a higher number of BWA F1 individuals and a greater number of gouts on the North American fir species, especially at higher initial inoculation densities, and reduced new growth in the North American host species. I also hypothesized that the European host species would be less affected by the initial inoculation densities. For the modified passive approach, I hypothesized that BWA F1 individuals would be found across all European and North American fir species, suggesting that the new modified passive approach to inoculate fir seedlings with BWA would be a viable method useful in testing seedlings for resistance or tolerance. However, I hypothesized that BWA crawler settling success would be higher in the North American fir species.

Statistical Analyses

Modified Passive Inoculation Approach

For data collected in the passive inoculation approach, generalized linear models were used in R Studio (Posit Team 2025) to measure the effect of BWA on the seedlings. The number of BWA individuals of the F1 generation was considered as a response variable, and I considered the main effects of species and root collar diameter, and their interaction. Root collar diameter was used as a proxy for seedling size, which could affect BWA settling success. I also considered the number of gouts and length of the new growth as response variables, and considered the main effects of the number of F1 BWA individuals, species, and diameter at root collar, and the interaction between species and root collar diameter.

Manual Inoculation Approach

Data from the manual inoculation approach were evaluated in three different collection manners: (1) seedings that were inspected during a timed 5-minute scan; (2) seedlings that were inspected without a time restriction; and (3) data combined from the previous two manners.

Timed Inspections

In the first manner (5-minute scans), I considered the main effects of initial BWA inoculation density and host fir species, and their interaction, on the number of F1 BWA individuals. I also included year as a random variable in a linear mixed-effects model. I also examined the main effects of the number of F1 BWA individuals, host fir species, and root collar diameter, and the interaction between host fir species and root collar diameter, on new growth length when also including year as a random variable. Both models were also examined by placing host fir species in one of three groups by range (European, Eastern North America, and Western North America) due to low replication in some fir species. I also considered if there were any differences in the number of F1 BWA individuals within the grand fir and subalpine fir families. For each species, I tested the main effects of initial inoculation density, SOW number (a proxy for the parent tree), and their interaction, on the number of F1 BWA individuals.

Untimed Inspections

Data from the second collection manner (untimed scans) were analyzed in a similar manner but without the inclusion of year as a random effect as untimed scans were only done in 2024 due to the time and labor involved. In this case, I used a generalized linear model to consider the effects of initial BWA inoculation density, host fir species, and their interaction on the number of F1 BWA individuals. The effects of the number of F1 BWA individuals, host fir species, and their interaction on new growth length was also examined. Both of these generalized linear models were also conducted using the three groups by their geographical range (European, Eastern North America, and Western North America) due to low replication at the individual species level.

Timed and Untimed Inspections Combined

When using data from both collection manners, I examined the main effects of the host fir species and initial inoculation density, and their interaction, on the number of gouts when including year as a random factor in a linear mixed-effects model. I also considered if there were any differences in the number of gouts within the grand fir and subalpine fir families. I tested the main effects of initial inoculation density and SOW number, and their interaction on the amount of gouts.

All response variables were transformed using $\log_{10}$ to meet the assumptions of normality. Linear mixed-effect models and generalized linear models were conducted in RStudio Version 2025.9.1.401(Posit Team 2025) using lmerTest (Kuznetsova et al. 2017). When appropriate, post-hoc tests were conducted using emmeans (Lenth 2026) to ascertain differences among the categorical predictor variables. Significance was assessed at α= 0.05.

Objective 2: Reconstructing BWA infestation history on the Gifford Pinchot National Forest

The second aim of the project is to use dendrochronological methods to construct the BWA infestation history on the GPNF. I also collected stand level data to uncover other factors, such as basal area and species composition, that could have contributed to resistance or tolerance to BWA. In late summer 2024, in collaboration with the USDA Forest Service Region 6, we revisited 13 individual trees from which seeds were collected in 1978 to 1995, from which some seedlings were used in Objective 1; these individual trees are henceforth referred to as parent trees and were comprised of subalpine firs. We also randomly selected one Pacific silver fir and three subalpine fir trees, henceforth referred to as nonparent trees. These trees are located throughout the GPNF (Fig. 9). Each parent and nonparent tree was marked as the plot center, and all living stems within 10 m of the plot center were identified to species and measured for the diameter at standardized height (DSH, 1.4 m from the ground). Any snags within this radius were also measured at DSH and species were identified when possible. A BWA infestation rating was given for each parent and nonparent tree based on the Hawksworth mistletoe rating (Hawksworth 1977). In this rating system, each third of the canopy was given a numerical rating from 0 to 4, where 0 indicated no visual detection of BWA damage, 1 for light infestation, 2 for moderate infestation, 3 for heavy infestation, and 4 for dead trees. Gouting, deformation, and defoliation were considered when rating each third. A plot infestation rating was also quantified based upon the BWA effects on regeneration, understory, and overstory trees. Ratings were recorded as light when only overstory trees had signs of BWA; moderate when overstory and understory trees had signs of BWA; or high when BWA was present on regeneration, understory and overstory trees.

Tree cores were also extracted at DSH from each parent and nonparent tree. Each tree was cored twice to increase the probability of detecting rotholz. Cores were extracted from the north and west side of the tree when possible, although some cores were extracted from the east or south side when the tree was obstructed or a safety hazard was present. Tree cores were allowed to dry for ~ one week, mounted on wooden runners, and then progressively sanded with a belt sander: 80,150, and 320 grit followed by hand sanding (60, 80, 120, 220, and 1,000 grit). Cores were scanned at 2400 DPI and annual tree ring widths were measured using CooRecorder (Cybis Elektronik 2024) and CDendro (Cybis Elektronik 2024). COFECHA (Holmes 1983) was used to cross-date the parent and non-parent trees to an existing chronology of subalpine firs located in the Gifford Pinchot National Forest available in the International Tree-Ring Data Bank (Peterson and Peterson 2019).

Plant root simulator (PRS) soil probes (WesternAg Innovations, Saskatoon, Saskatchewan, Canada) were deployed in 6 parent tree sites and 4 nonparent tree sites. These probes are designed to measure soil nutrients in various ecosystems, such as grasslands, agriculture, wetlands, and forests, using an ion-exchange resin membrane that is inserted into the soil (Littke et al. 2024). The cation probe measures positively-charged cations (NH_4^+, K^+, Ca^{2+}, Mg^{2+}, etc.) by using a negatively-charged membrane while the anion probe measures negatively-charged anions using a positively-charged membrane (NO_3^-, $H_2PO_4^-$, SO_4^{2-}, etc.) (Fig. 10) (WesternAg Innovations 2018). At each site, a total of 16 PRS probes were placed 5m (8 probes) and 10m (8 probes) from the selected parent or nonparent tree in all four cardinal directions (N, W, S, and E). In each cardinal direction, one cation probe and one anion probe was deployed (Fig. 10). Probes were buried ~12 cm into the ground. After ~6 weeks, PRS probes were collected, cleaned with a brush and DI water, and sent to WesternAg Innovations for nutrient analysis.

Hypotheses

I hypothesized that initial signs of BWA infestation on the Gifford Pinchot National Forest will be detected in the tree core record, dating to at least the late 1950s when it was first confirmed to be present. I also hypothesized that there is an environmental influence on the parent trees' ability to withstand multiple years of BWA infestation. Specifically, I expect to observe a greater infestation of BWA, as quantified using tree and plot ratings, in stands with a higher basal area in suitable BWA host trees, as measured using plot-level data.

Statistical Analyses

Host tree ring widths were analyzed as a proxy to discern if there were increases in growth that may suggest the presence of rotholz. After cross-dating, and because host trees were cored twice, the core with the shortest time series was removed. A total of 15 tree cores were used in this analysis; one of the non-parent host trees was also removed because it was from a Pacific silver fir while the rest were from subalpine fir. Tree ring measurements were detrended using a negative exponential model in R Studio (Posit Team 2025) according to:

$$\text{ring width (mm)} = a \times \exp^{(b \times age)} , \qquad \text{(Eq. 1)}$$

where a and b are fitted parameters (Fritts 1976). The model was used to determine the predicted tree ring widths by year, which were then subtracted by the observed tree ring widths to calculate the residuals. The residuals (i.e., detrended for age-related growth trends) were graphed to demonstrate any unusual increases in tree ring widths over time, from 1853 to 2023.

The plot data collected from the Gifford Pinchot National Forest were analyzed using a generalized linear model in R studio (Posit Team 2025) to measure the effect of environmental factors on the host infestation rating. Host infestation ratings were converted from the three number rating to low or moderate/high by averaging the three numbers with anything equal to two or higher being moderate/high and anything less than two being low; therefore, the generalized linear model was run using the binomial distribution. The main effects I considered were plot basal area of live trees (m^2 ha^{-1}), the proportion of live basal area of true fir, and elevation. A total of 14 plots were used; three plots were removed from analysis because the parent tree was dead. Of the living parent and nonparent plots, only 8 had soil probes, therefore soil nutrients were not considered in the generalized mixed model due to insufficient replicates. Furthermore, several plots had nutrient levels below detection levels; instead, a descriptive summary of the nutrient data from the soil probes is presented in Table 2.

Results

Objective 1: Testing for resistance or tolerance to balsam woolly adelgid

Modified Passive Inoculation Approach

Of the 280 seedlings that were inoculated with BWA using the modified passive approach, 159 were examined and used in statistical analysis. The remaining seedlings either died or were too moldy to be processed following storage. Of the 159 seedlings, 147 were observed with gouting, and F1 BWA individuals were observed on 141 (Table 1). The highest and lowest mean number of gouts was observed in Canaan fir (102.12, SE=7.53) and Turkish fir (1.89, SE=0.64), respectively (Table 1). The highest and lowest mean number of BWA individuals was on Fraser fir (25.03, SE=2.06) and Turkish fir (2.44, SE=0.68), respectively (Table 1).

The number of gouts was significantly affected by the species (F=239.0; df=6, 152; P<0.01) and diameter at root collar (F= 54.9; df=1,151; P<0.01), while the interaction effect was not significant (F=1.6; df=6,145; P=0.16). Post hoc tests revealed that the two European species, Turkish and Nordmann fir, had a significantly lower number of gouts relative to the three Eastern and two Western U.S. species (Fig. 11). Also, with a larger diameter at root collar, there was more gouting on the seedlings (Fig. 12).

When examining the number of F1 BWA individuals, the effect of species was significant (F=43.4; df=6,152; P<0.01). There was no significant effect of diameter at root collar (F=0.02; df=1,151; P=0.9) (Fig. 13) or its interaction with species (F=1.48; df=6,145; P=0.19). Post hoc tests revealed significant differences among the species (Fig. 14) and in general, there were more F1 BWA individuals on the Eastern U.S. species.

Seedling new growth length was significantly affected by the number of F1 BWA individuals (F=187.0; df=1,157; P<0.01), species (F=33.7; df=6,151; P<0.01), and root collar diameter (F=26.9; df=1,150; P< 0.01). As the number of F1 BWA individuals increased, the length of the new growth of the North American species also increased while the European species' new growth decreased (Fig. 15). A similar pattern was noted when considering diameter at root collar by new growth length (Fig. 16); as diameter increases the length in new growth increases for North American species while the opposite pattern was observed in the European species. Among species, post hoc tests revealed significant differences among the species and that the European species and subalpine fir had shorter new growth length (Fig. 17).

Manual Inoculation Approach

Timed Inspections

Of the 11 seedlings examined in a timed inspection that were inoculated with only one female BWA, nine seedlings had signs of gouting (81.8%) and two were observed with F1 individuals (18.2%).

In five-minute inspections to enumerate the number of F1 BWA individuals, there was a significant effect of species group (i.e., European, Eastern North America, and Western North America; F=4.05; df=2,131.77; P=0.02). Post hoc tests revealed that the Eastern North American species had significantly more F1 BWA individuals than seedlings from Western North American and Europe (Fig. 18). When considering the effect of specific species, there was a significant effect on the number of F1 BWA individuals (F=15.24; df=5,132.28; P<0.01), with Eastern North American species having more (Fig. 19). There was no significant effect of initial density (Fig. 20), or its interaction with species group, or species, when either species group or species was included in the model (all P>0.11).

The length of the new growth was significantly affected by species group (F=5.5; df=2,115.1; P<0.01), diameter at root collar (F= 34.6; df=1,128.21; P<0.01), and their interaction (F= 7.3; df=2,123.75; P<0.01). Post hoc tests revealed that the Western North American group had significantly shorter lengths in new growth than the Eastern North American and European groups (P-values 0.007 and 0.001, respectively) (Fig. 21). Similar effects on new growth length was also observed when including species as a main effect (F=3.49; df=5,126; P<0.01) along with diameter at root collar (F=13.6; df=1,126; P<0.01), and their interaction (F= 3.8; df=5,126; P<0.01). Post hoc tests revealed that there was a significant difference among the species with Canaan fir having the longest new growth (Fig. 22).

The number of F1 BWA individuals was not significantly different among grand fir families (P=0.39), affected by the interaction (P=0.14) or affected by the initial inoculation density (P=0.09). The number of F1 BWA individuals was also not significantly affected by the initial inoculation density ($P=0.22$), or different among subalpine families ($P=0.97$) or affected by the interaction ($P=0.73$).

Untimed Inspections

A total of 47 seedlings inoculated with one female BWA were examined in the untimed inspections. Of the 47 seedlings, 29 had signs of gouting (61.7%) and 13 were observed with F1 BWA individuals (27.7%).

In untimed inspections used to enumerate the number of F1 BWA individuals, there was a significant effect of species group ($F=5.59$; $df=2,78$; $P=0.005$), and a significant interaction between initial inoculation density and species group ($F=3.57$; $df=2,76$; $P=0.033$). Post hoc tests revealed that fir hosts native to Eastern North America had significantly more from those native to Western North America ($P<0.01$), while European species did not have significantly more or less from Eastern and Western North America ($P=0.15$, $P=0.17$, respectively) (Fig. 23).

When including the effect of individual species on the number of F1 BWA individuals in a generalized linear model, there was a significant effect of species ($F= 5.61$; $df= 6,74$; $P< 0.001$), but no significant effect of the interaction between species and the initial density ($F=2.23$; $DF=6,68$; $P=0.05$). Post hoc tests revealed significant differences among the species and that there was a higher density of BWA F1 generation on Nordmann and Canaan fir (Fig. 24).

Consistent with the timed inspections, the length of the new growth was also affected by species group ($F=79.42$; $DF=2,78$; $P<0.001$) and the diameter at root collar ($F=11.33$; $df=1,77$; $P=0.001$). Post hoc tests revealed that the Eastern North American group had significantly longer new growth from the European ($P=0.029$) and Western North American ($P=0.01$) groups (Fig. 25). Similar results were seen at the individual species level where the length of new growth was significantly affected by the species ($F=30.87$; $df=6,74$; $P<0.001$), and the interaction between species and diameter at root collar ($F=2.75$; $df=6,67$; $P=0.019$). Post hoc tests showed that Balsam, Canaan, and Fraser fir had longer new growth than the other species (Fig. 26).

Timed and Untimed Inspections Combined

The number of gouts was significantly affected by the initial inoculation density (F=12.51; df=1,209.14; P<0.001), species (F=13.4, df=6,209.06; P<0.01), and the interaction between initial inoculation density and species (F=5.91; df=6,209.11; P<0.001). As initial inoculation density increased, the number of gouts found on the species increased (Fig. 27). Post hoc tests revealed that subalpine fir had more gouts and the European species had the least (Fig. 28).

The number of gouts was significantly affected by the grand fir families (F= 2.91; df=4,36.04; P=0.03), the initial inoculation density (F= 7.22; df=1,39.99; P=0.01) (Fig. 29) but not the interaction between family and initial inoculation density (F=1.97; df=4,31.2; P=0.12). However, post hoc tests revealed no significant differences among the grand fir families (Fig. 30).

The number of gouts was significantly affected by the subalpine fir families number (F= 3.45; df=6,50.03; P>0.01), the initial inoculation density (F= 21.6; df=1,56; P>0.01) (Fig. 31), but not the interaction between family and initial inoculation density (F=1.24; df=6,43.32; P=0.31). Post hoc tests revealed that the SAF-21 family had significantly less gouts than the SAF-3 family (Fig. 32).

Objective 2: Reconstructing BWA infestation history on the Gifford Pinchot National Forest

Parameters for the negative exponential model (Eq. 1) are shown in Table 3. Using the residuals based on the negative exponential model (Fig. 33A), there was a visual increase in tree ring widths in the 1950s to early 1970s (Fig. 34B), which coincides with prior reports regarding the initial infestation on the Gifford Pinchot National Forest (Johnson and Wright 1957).

When examining the plot level data on the binary response of BWA infestation rating (low vs medium-to-high), there was a non-significant trend between the level of infestation on host trees and the live basal area (Z= -1.675; P=0.09); as plot basal area increases, the level of infestation decreases (Fig. 25). There was no significant effect of elevation (Z= -0.49; P=0.63) or the proportion of basal area in fir (Z= 0.91; P=0.37) on the host tree infestation rating (Figs. 35 & 36).

Discussion

Objective 1: Testing for resistance or tolerance to balsam woolly adelgid

The new modified passive approach was successful in infesting seedlings with BWA. Results from this approach are similar to those of the manual inoculation approach, reinforcing its effectiveness in infesting seedlings. Due to its relative simplicity relative to the method of suspending infested bolts (Newton et al. 2011), this method provides a good option for managers and researchers in efforts to screen seedlings for BWA resistance under time and budget constraints.

As hypothesized, both European species, Turkish and Nordmann fir, had significantly less gouts in both the modified passive and manual inoculation approaches relative to the Eastern and Western North American species. In the combined timed and untimed inspections, subalpine fir had significantly more gouts than all the other species, while the European species had the least. The results reinforce the known range of susceptibility of the European and North American fir species to BWA (Newton et al. 2012, Balch 1952, Hain 1991, Mitchell 1966).

The modified passive approach results also demonstrated that there was a significant trend in which seedlings from North America with larger root collar diameters had more gouts; however, no trend was seen in the European species (Fig. 12). This observation could be due to how each of the species compartmentalizes BWA feeding, with the European species potentially allocating resources to defend, while the North American species have not evolved adequate defenses to BWA.

Not surprisingly, as the initial inoculation of BWA on the seedlings increased, so did the subsequent number of gouts observed on the seedlings. However, the rate of this increase was higher for the Eastern and Western North American species, while the rate on the European species only incrementally increased (Fig. 27). This observation further illustrates the importance of evolutionary history and how evolutionarily native hosts react differently to BWA than evolutionarily naive species.

Interestingly, the Eastern North American species had significantly more F1 BWA individuals relative to Western North American and European species, regardless of the inoculation approach. This observation was also not explained by their larger root collar diameters as there was no significant effect of root collar diameter on the number of F1 BWA individuals (Fig. 13). This was also observed from the manual inoculation study where seedling size also did not play a significant role, and where all seedlings were inoculated with the same number of BWA individuals.

The length of the new growth was significantly affected by the species and root collar diameter for both the modified passive and manual approaches. Eastern North American species tended to respond to BWA feeding by producing the longest new growth relative to Western North American and European species, regardless of the inoculation approach (Figs. 17, 21, 22, 25, & 26). Also, as the number of F1 BWA individuals increased, so did the length of the new growth on the North American species, while the opposite was seen on the European species (Fig. 16). Insect herbivory is generally associated with reduced plant growth as plant resources are instead allocated to defense (Herms and Mattson 1992). This association was observed in the European species, which as the BWA evolutionary-native hosts could be allocating more resources into defense instead of growth. The North American fir species, which tended to produce longer new growth, may not be allocating sufficient resources to defense, thereby allocating resources to growth; another possibility could be that BWA pressure may stimulate shoot expansion that the North American fir species lack adaptations to manage.

There were no significant differences within the grand fir and subalpine fir families regarding the number of F1 BWA individuals suggesting that there may not be any genetic resistance/tolerance to BWA. However, this result could be due to a low number of replicates within each family. This could explain why grand fir family had a significant effect on the number of gouts, even though post hoc tests did not reveal any differences among the families (Fig. 30). On the other hand, post hoc tests did reveal a difference between SAF-21 and SAF-3 (Fig. 32) in which the former had less gouts. However, due to the low number of replicates, further screening should be done to determine if any families are genetically resistant/tolerant to BWA.

Objective 2: Reconstructing BWA infestation history on the Gifford Pinchot National Forest

The increase in tree ring widths during the 1950s (Fig. 33B) coincides with Johnson and Wright (1957), who first detailed the presence of BWA in the Pacific Northwest in 1954. The increase in tree ring width also persisted to the 1970s, demonstrating the potential long-term effect of BWA feeding in the GPNF. However, even though there is an increase in tree ring width that coincides with the first report (Johnson and Wright 1957), a more complete dendrochronology analysis, in which climate signals are removed from cores, would be needed to further refine BWA invasion history in the GPNF (Leef 2019).

Despite the lack of significance of the plot-level variables on BWA infestation ratings in the GPNF, there was a non-significant trend; as total plot basal area increased, the BWA infestation rating decreased (Fig. 34). This is an interesting observation as it would be expected that in plots with higher basal area, resources would be more limited and more competition would lead to higher levels of infestation due to host tree stress. There was also an insignificant trend between the proportion of basal area in fir and the BWA infestation rating; as the proportion increased, so did the probability of a higher infestation rating (Fig. 36). Despite the lack of statistical significance, which could be due to a relatively small sample size given the attention to the parent trees in this study, it is intuitive that with increased fir basal area, the likelihood of a higher BWA infestation rating increases. Silvicultural practices could be a useful management strategy to reduce BWA infestation levels in this National Forest. For example, silvicultural strategies have been proposed to address the invasion of the congener hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), in Eastern North America (Muzika 2017, Fajvan and Hille 2023).

Chapter 3. Conclusions

The research conducted for my thesis is an important step to furthering our knowledge on BWA in the PNW. The primary objective of my thesis to test seven different fir species for their susceptibility to BWA offered various interesting results. Firstly, the modified passive approach was a very successful method to inoculate seedlings with BWA in support of studies that seek to screen seedlings to BWA resistance and tolerance, making it a viable option for future researchers. It was also observed that the European species, Turkish and Nordmann fir, had less gouts than the other five species, which was anticipated given the evolutionary history between BWA and these host species. Interestingly, the European species had a different growth reaction to BWA, as proxied by the length of new growth, than the North American species, which may be due to resource allocation. Moreover, the Eastern North American species, Canaan, Fraser, and Balsam fir, had longer new growth and more F1 BWA individuals, which was also unrelated to their size. This suggests that these Eastern North America species are more susceptible to BWA than their congeners in Western North America, even though both are considered to be evolutionarily naive. More research would be helpful in furthering determining if seed stock collected from the parent trees in the GPNF are resistant and tolerant to BWA. This remains an important question for land managers overseeing forested lands affected by BWA, and for informing reforestation efforts. Due to time constraints, particularly given the tedious task of counting BWA on seedlings, a full analysis for the second objective of my thesis (i.e., reconstructing the infestation history of BWA in the GPNF using dendrochronology) was not feasible. However, the residuals from the negative exponential growth model suggested that there was an increase in tree ring widths in the 1950s, which coincides with the first reports of BWA in Washington. A full dendrochronology analysis will be helpful in better linking the tree core record in the GPNF with the history of BWA.

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Tables

Table 1. Number of gouts, and number of FI BWA individuals by fir species from the modified passive inoculation experiment.						
	Gouts			**F1 BWA Individuals**		
Species	**Mean**	**SE**	**n**	**Mean**	**SE**	**n**
Fraser Fir	93.1	4.8	32	25.0	2.1	32
Balsam Fir	101.7	10.9	25	17.5	2.2	25
Canaan Fir	102.1	7.5	25	20.8	1.8	25
Subalpine Fir	36.5	3.7	25	3.0	0.6	18
Grand Fir	78.0	12.7	22	5.4	1.0	22
Turkish Fir	1.9	0.6	10	2.4	0.7	12
Nordmann Fir	2.7	1.4	8	3.8	1.9	7
Total			**147**			**141**

Table 2. Statistical summary of N, P. and K as measured from soil probe data deployed in GPNF plots. All probes were placed around a center live host tree.							
		Nutrient Supply (mg/10 cm^2/burial length					
		Nitrogen (N)		**Phosphorus (P)**		**Potassium (K)**	
Tree Status	**# of Plots**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
High	5	2.04	0.29	26.23	3.87	593.33	79.84
Low	3	3.58	0.63	18.35	3.79	1022.67	155.35

Table 3. Parameter estimates from the negative exponential model (Eq. 1) used to detrend tree ring measurements.

	Estimate	**Std. Error**	**t-value**	**Pr(>|t|)**
a	3.915584	0.083081	47.13	<2e-16*
b	-0.01527	0.000609	-25.08	<2e-16*

Figures

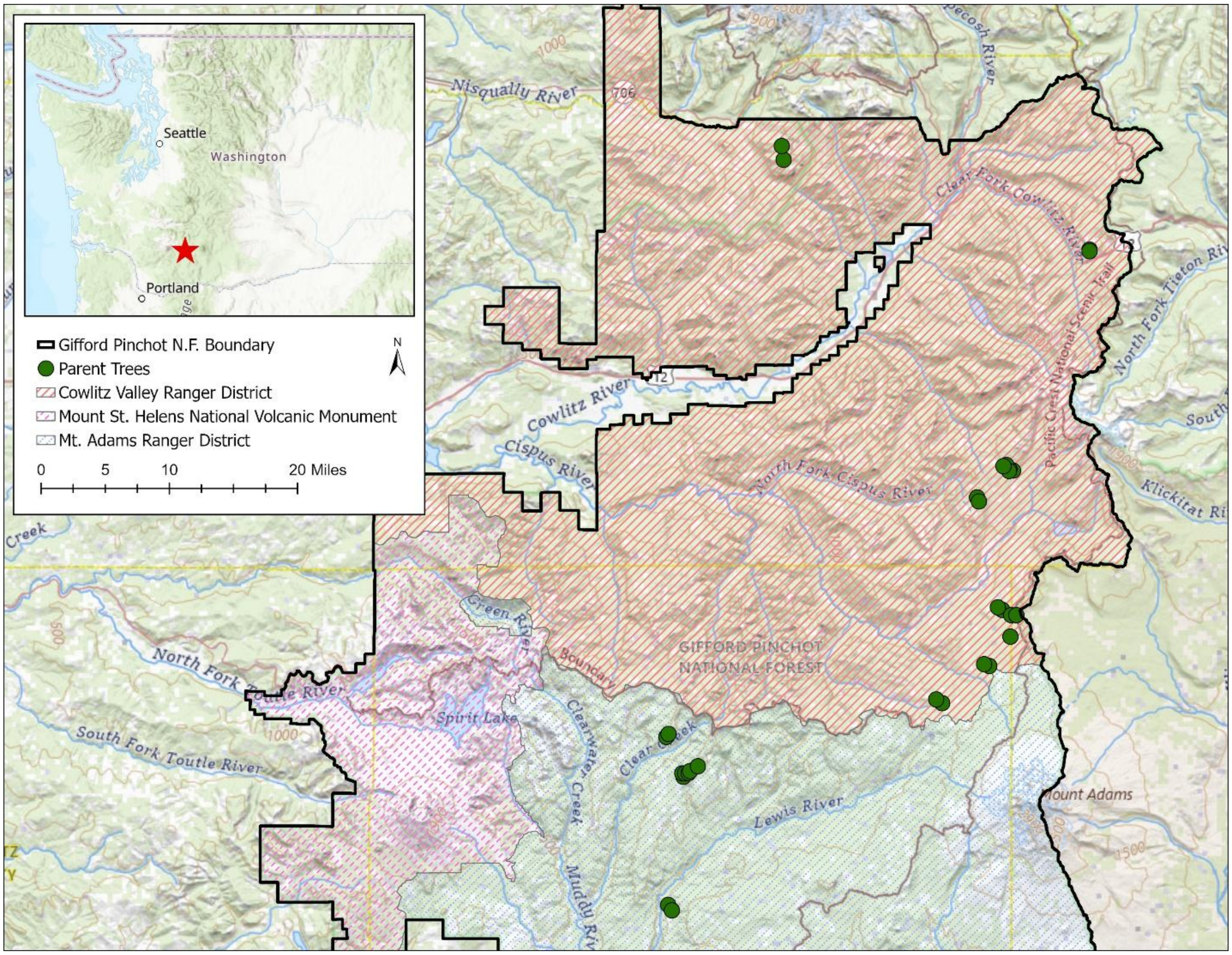

Figure 1. Location of sampling plots with subalpine and grand fir parent trees in the Gifford Pinchot National Forest, Washington, USA.

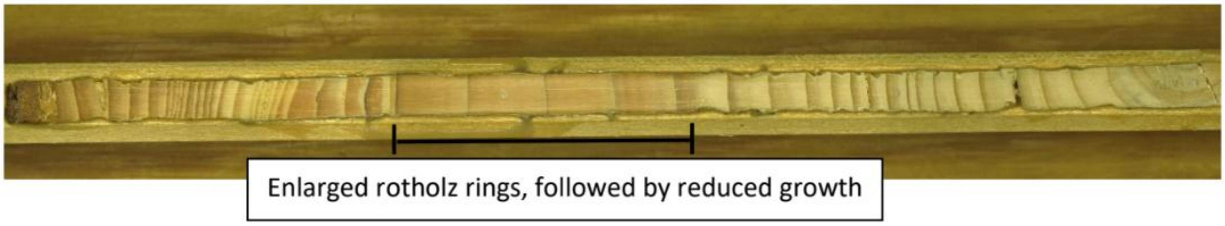

Figure 2. Example of Rotholz on a tree core in response to BWA feeding. Image reproduced from Leef (2019).

Figure 3. Photo of the modified passive inoculation approach conducted at the Washington State University Puyallup Research and Extension Center. The white circles illistrate the seedlings grouped under the infested Balsam fir trees.

Figure 4. Photo of a seedling used in the manual inoculation approach. The arrows demonstrate how infested branches were attached to the seedlings.

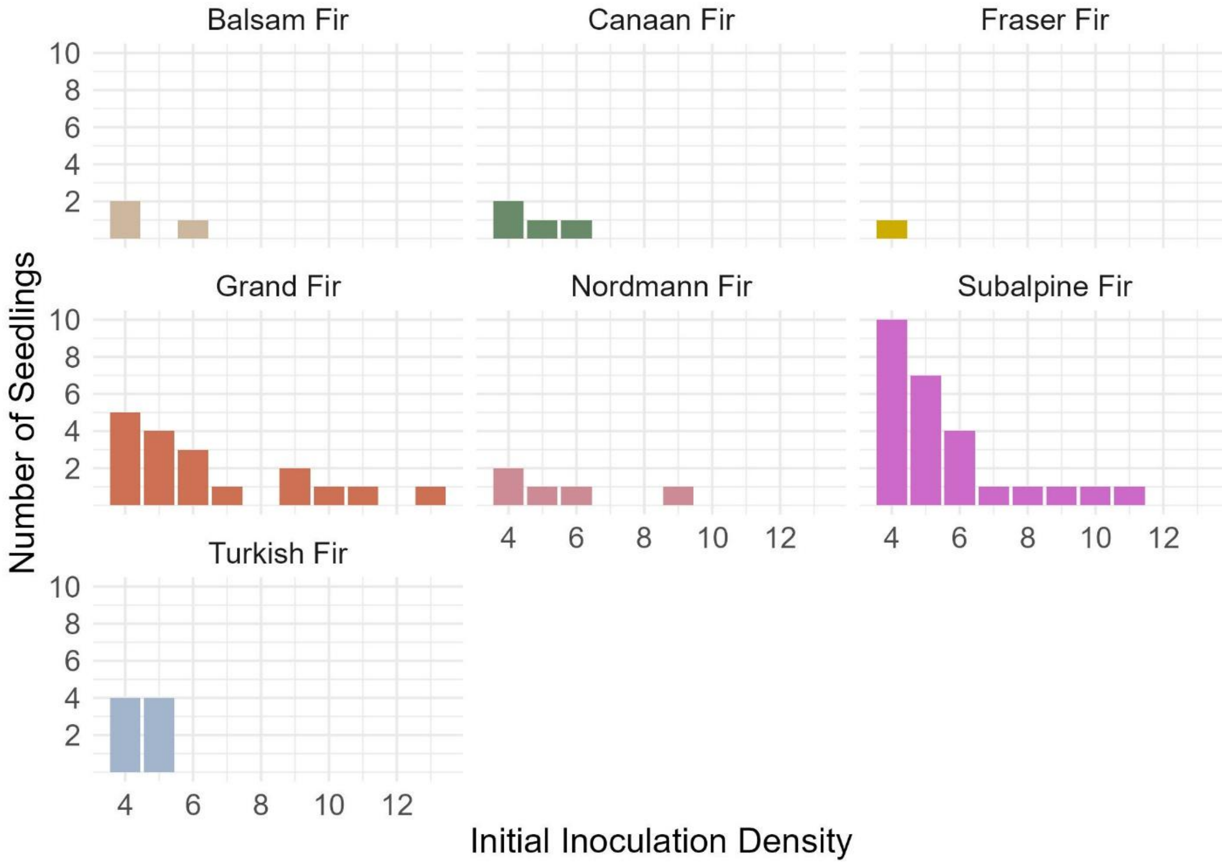

Figure 5. Histograms showing the initial inoculation density of BWA from 2024, which was used to inform the inoculation densities needed in 2025.

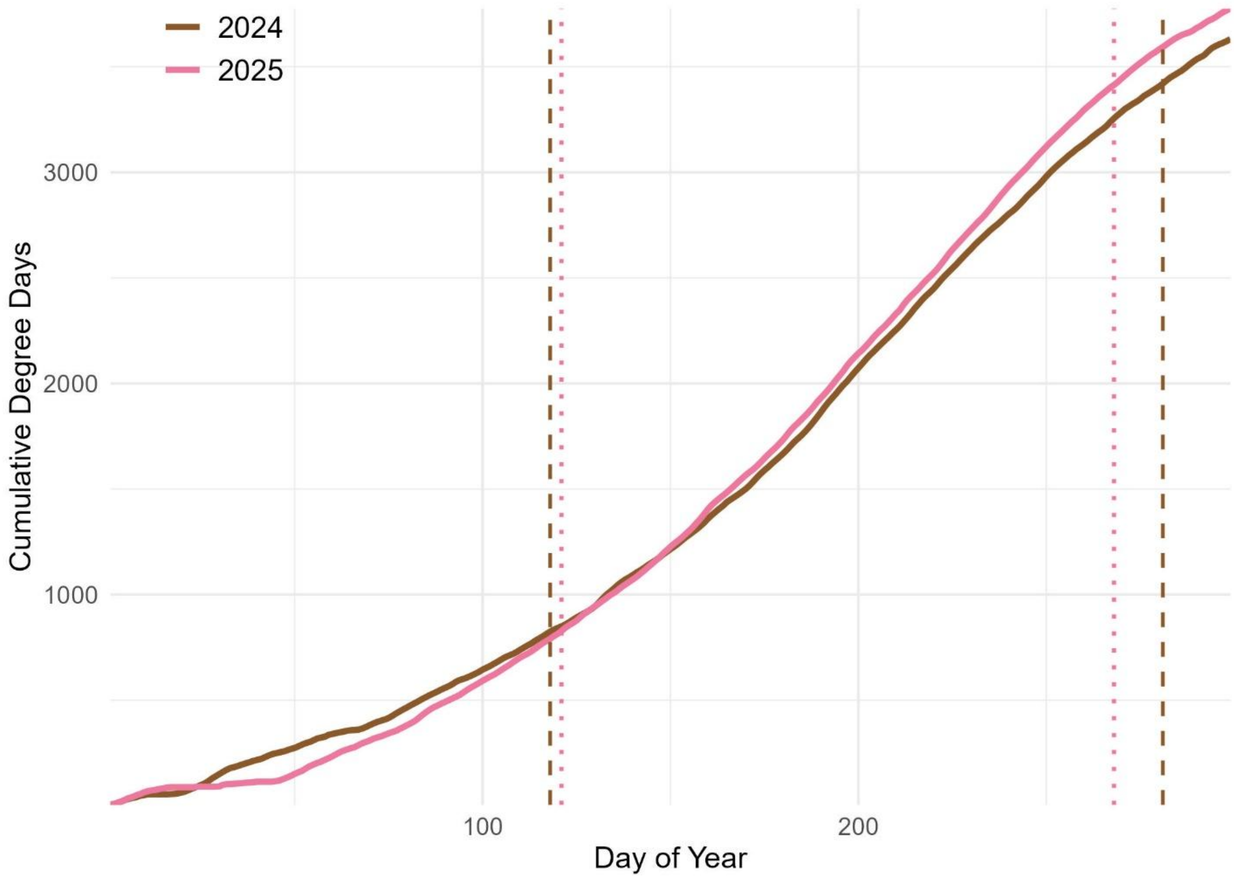

Figure 6. Cumulative degree days for 2024 and 2025 (base threshold = 0 ◦C) from the Washington State University Puyallup Research and Extension Center. The dash (2024) and dotted (2025) vertical lines represent the average day of BWA inoculation and when seedlings were subsequently cut for data processing.

Figure 7. Photo demonstrating how new growth length was measured on each seedling when processed.

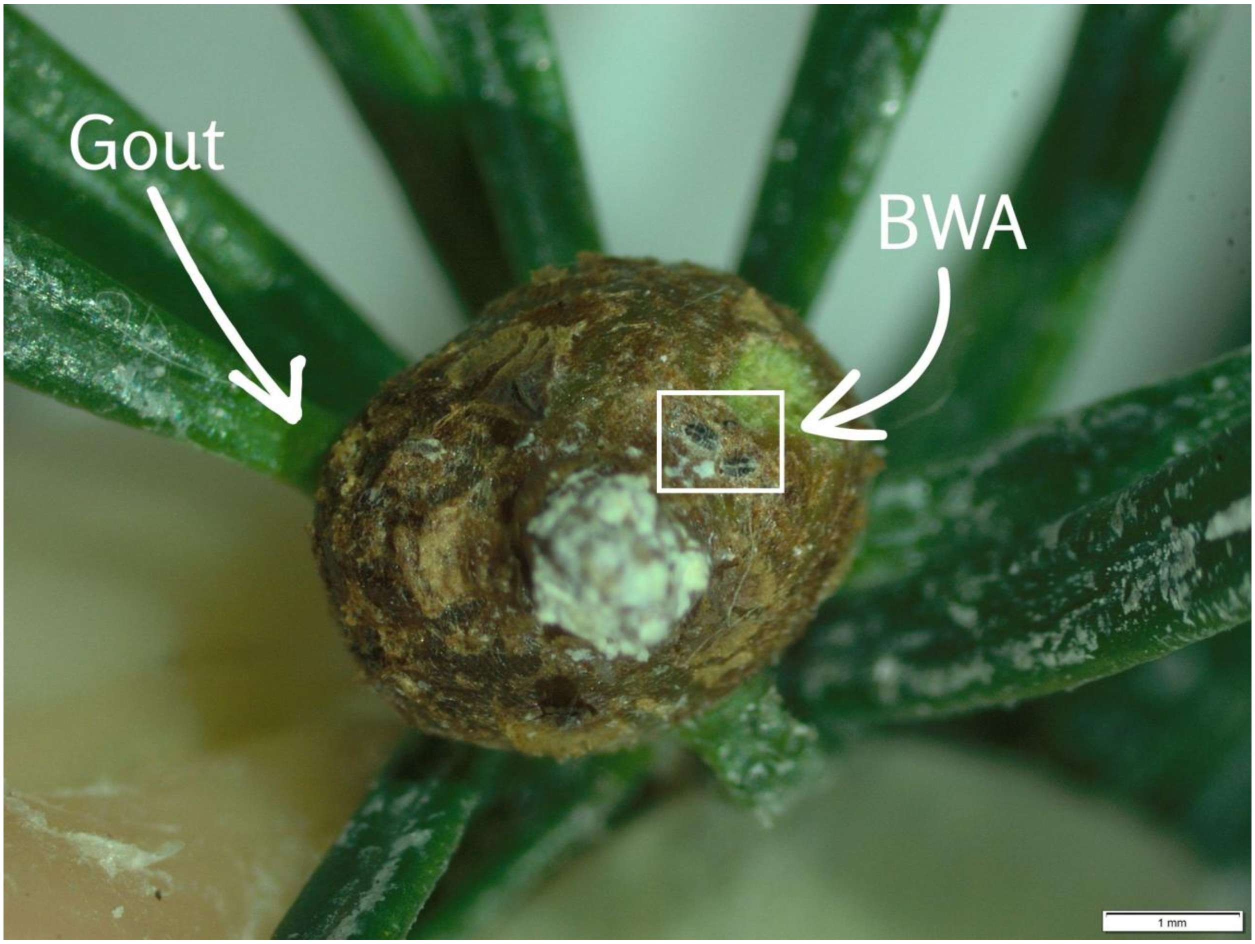

Figure 8. Image of a branch with a gout and two F1 BWA individuals.

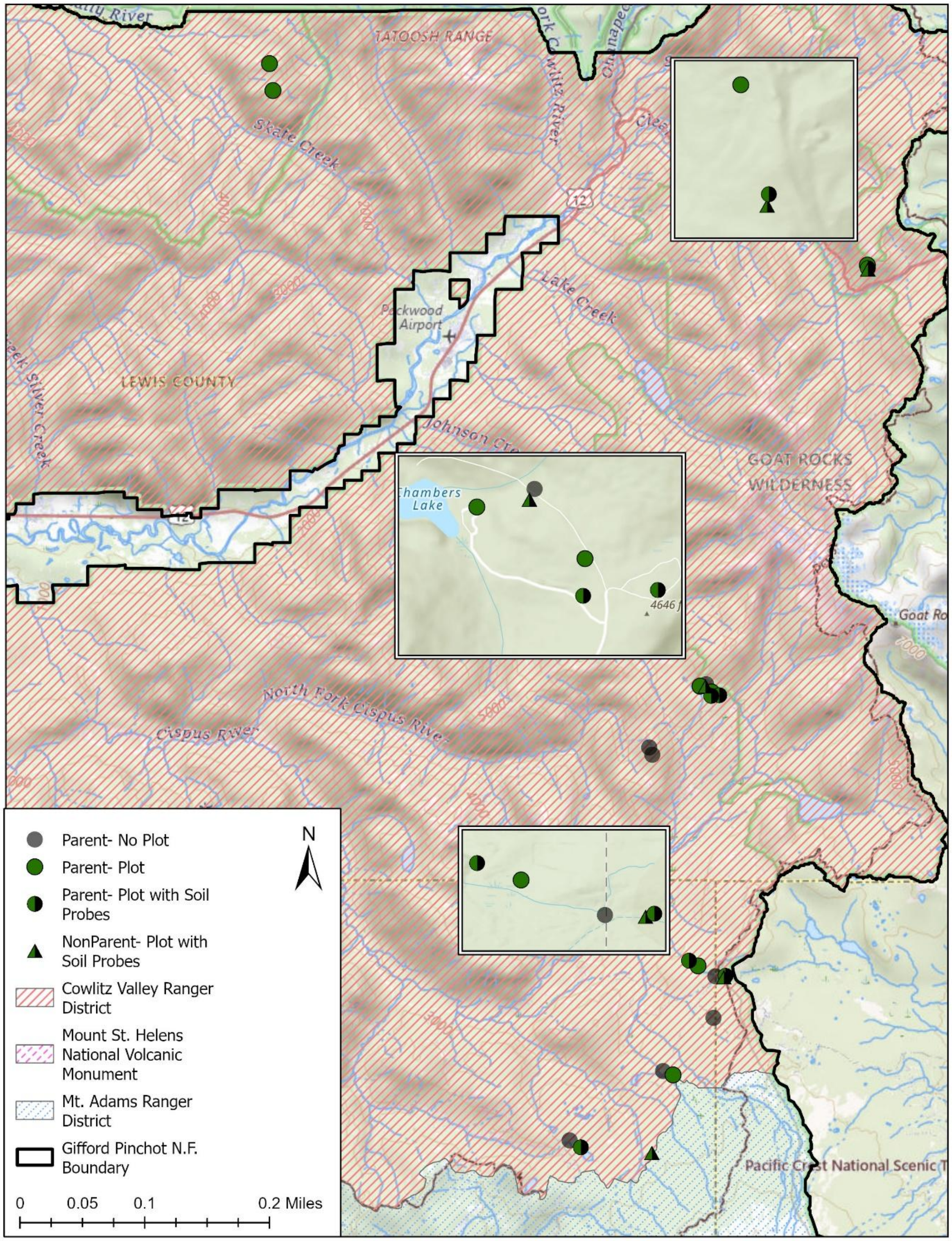

Figure 9. Location of the subalpine fir parent trees on the Gifford Pinchot National Forest, Washington, USA, that re-visited in Summer 2024, along with the non-parent host tree plots.

Figure 10. Cation (purple with pink flagging) and anion soil probes (orange) used to quantify soil nutrients at plots on the Gifford Pinchot National Forest, Washington, USA (cf. Table 2).

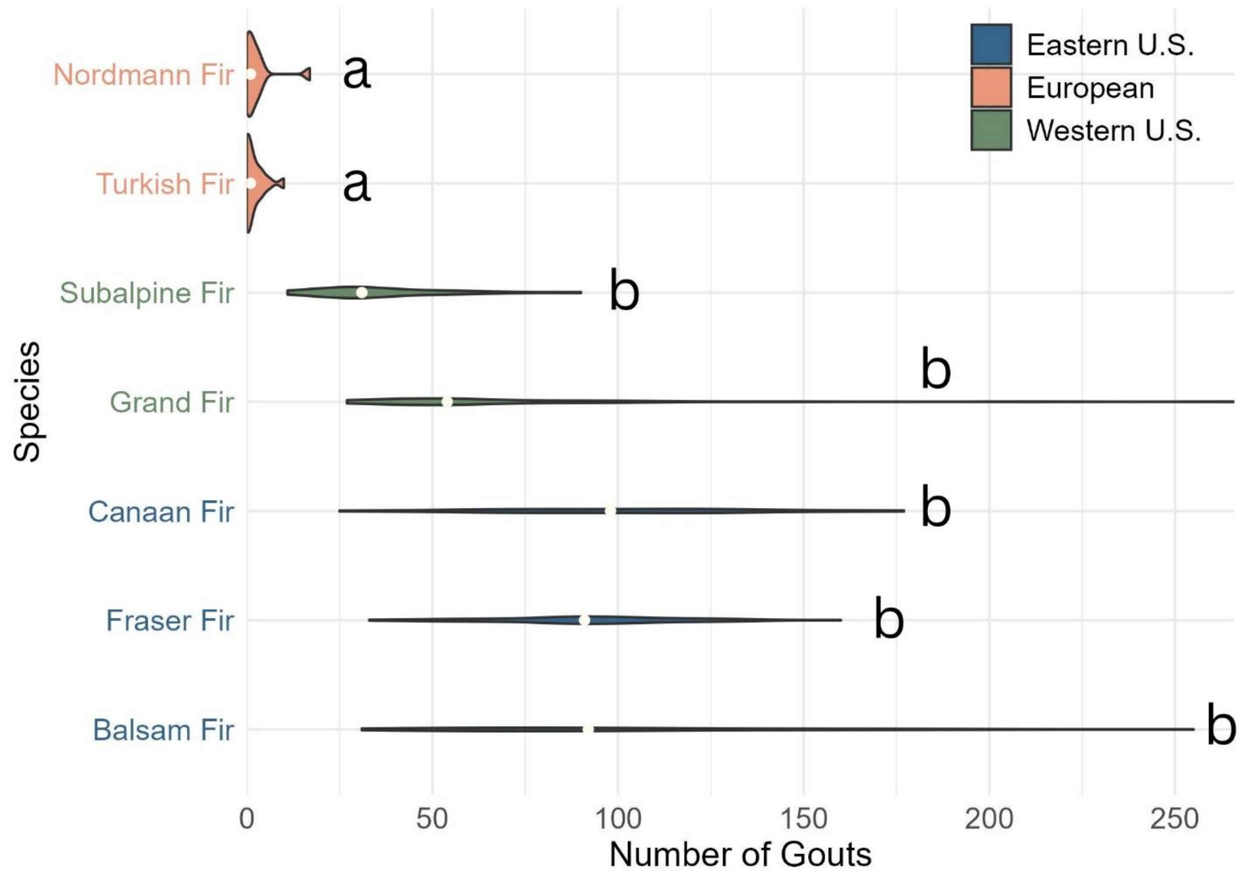

Figure 11. The number of gouts by species quantified from the modified passive approach in which seedlings were placed under BWA-infested Balsam fir at the Washington State University Puyallup Research and Extension Center in 2024. Different letters denote significant differences (α = 0.05).

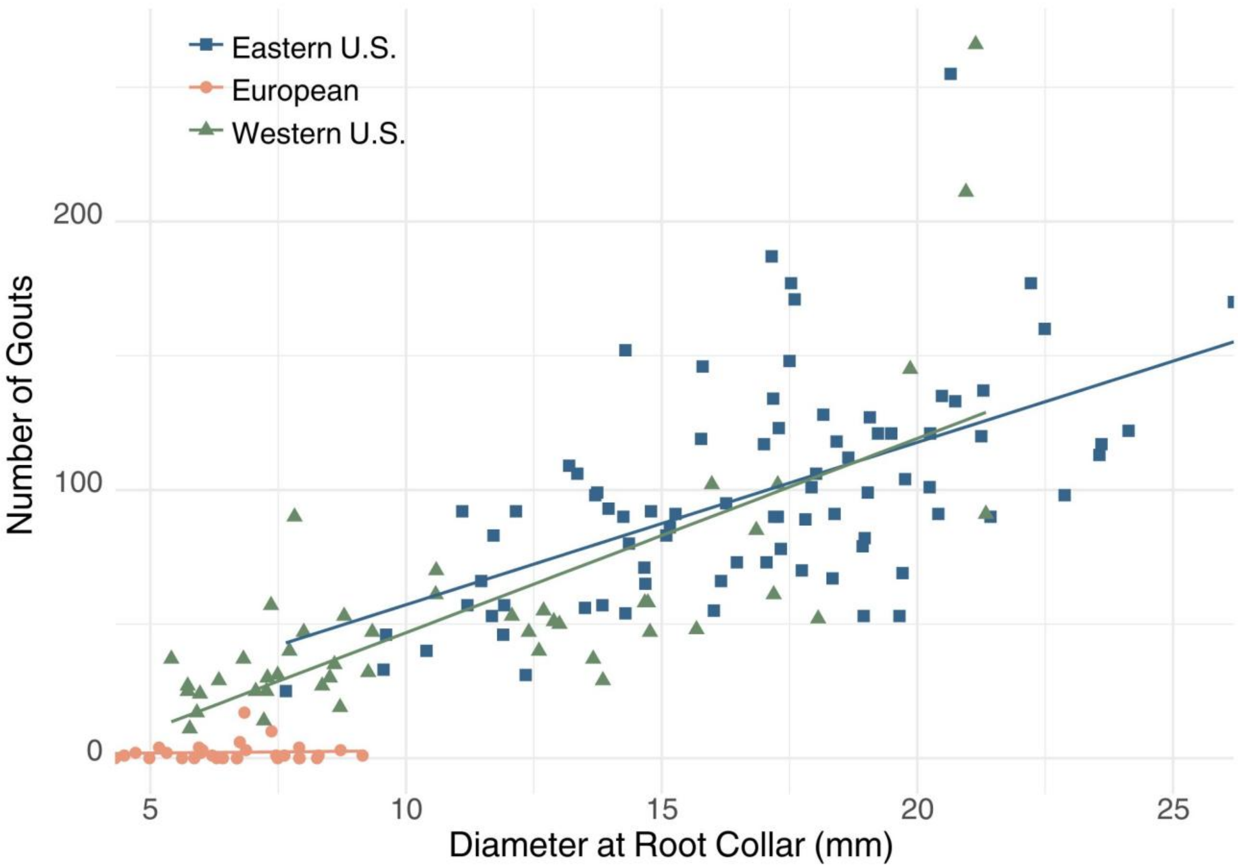

Figure 12. Number of gouts colored by species group as a function of seedling diameter at root collar (mm) quantified from the modified passive approach in which seedlings were placed under BWA-infested Balsam fir at the Washington State University Puyallup Research and Extension Center in 2024.

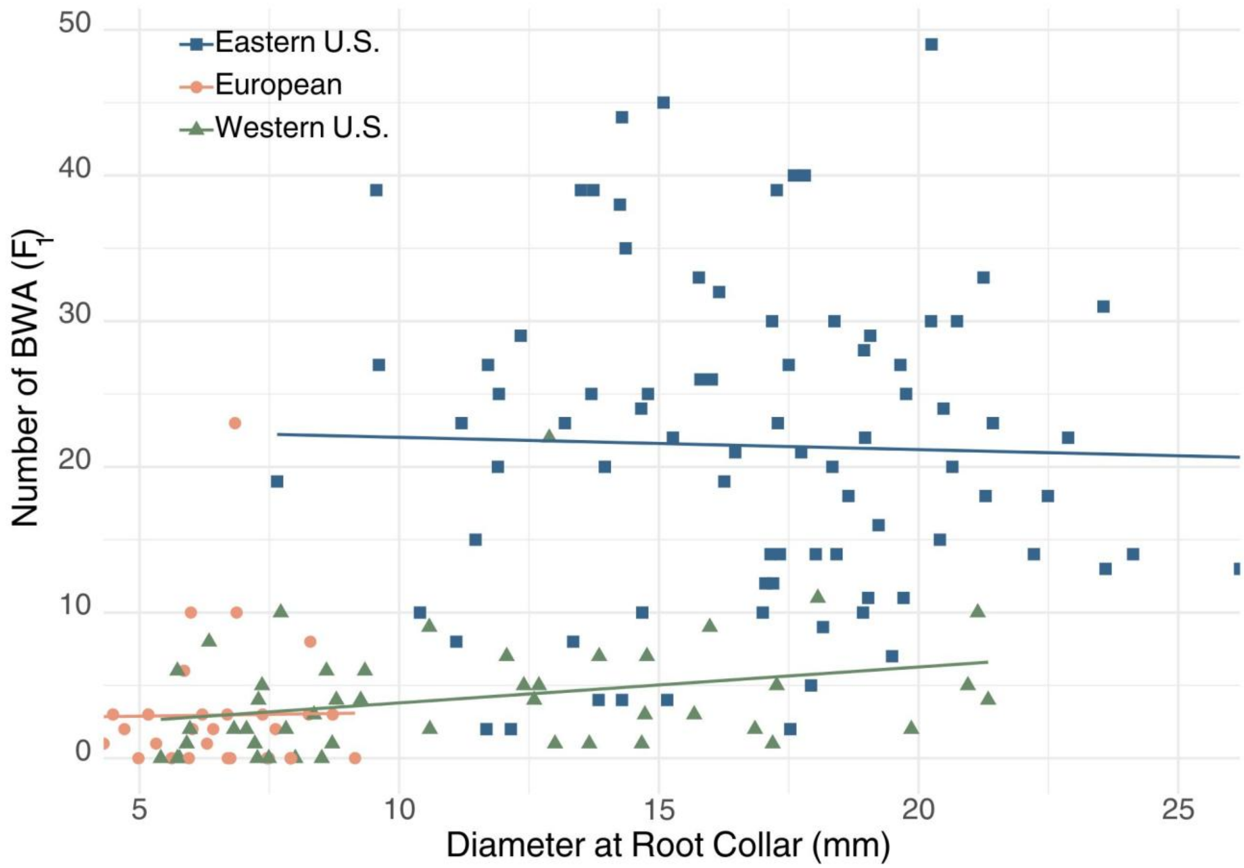

Figure 13. Number of F1 BWA individuals by species group as a function of seedling diameter at root collar (mm) quantified from the modified passive approach in which seedlings were placed under BWA-infested Balsam fir at the Washington State University Puyallup.

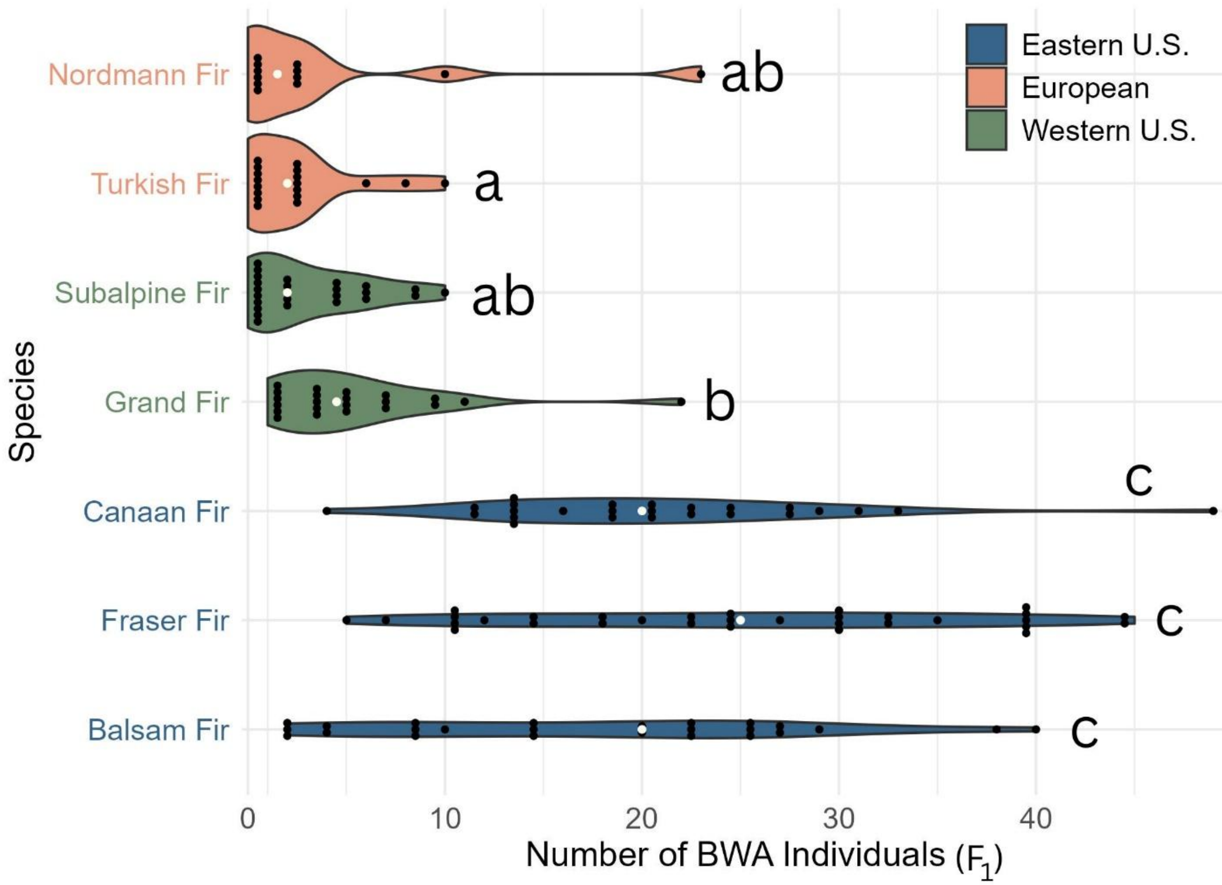

Figure 14. The number of F1 BWA individuals by species quantified from the modified passive approach in which seedlings were placed under BWA-infested Balsam fir at the Washington State University Puyallup Research and Extension Center in 2024. Different letters denote significant differences (α = 0.05).

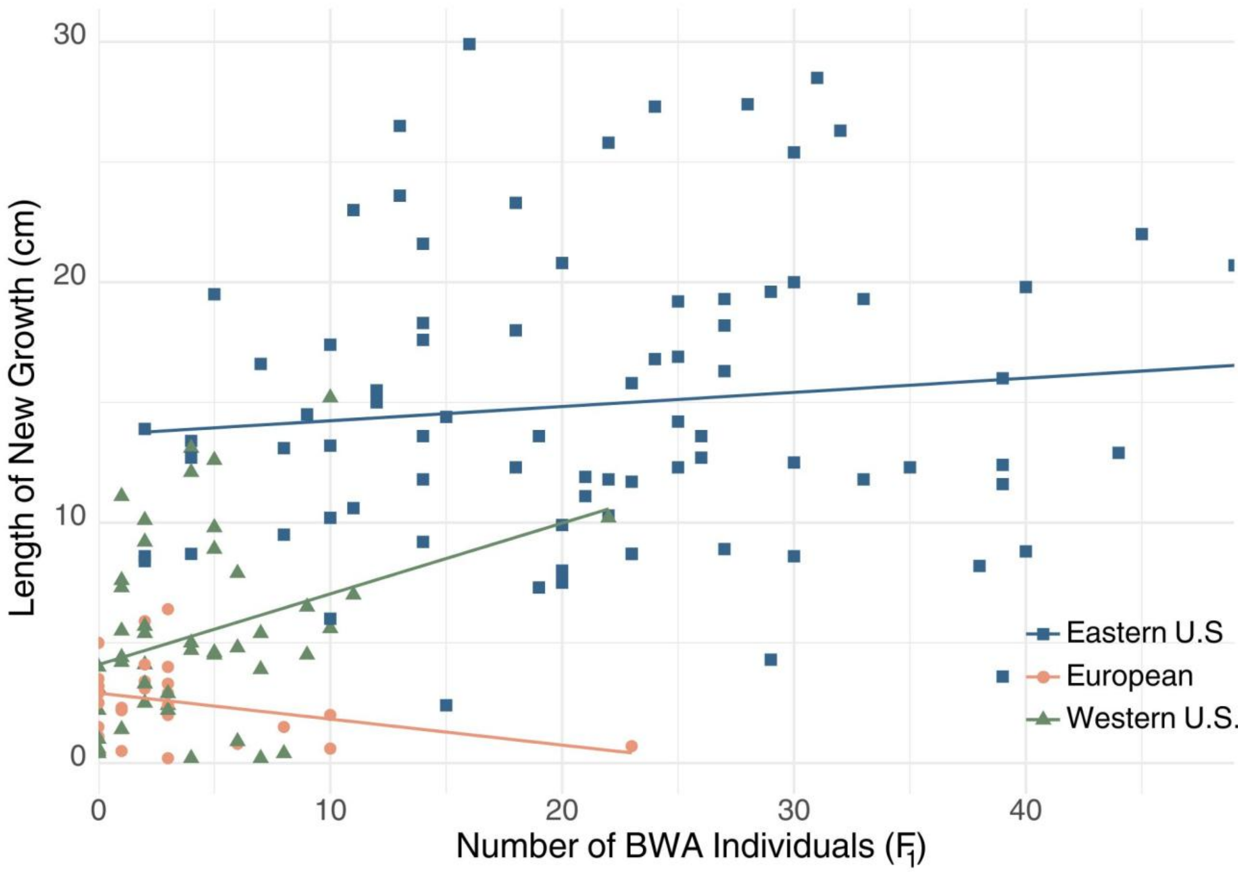

Figure 15. Length of new growth (cm) as a function of the number of F1 BWA individuals colored by species group, quantified from the modified passive approach in which seedlings were placed under BWA-infested Balsam fir at the Washington State University Puyallup Research and Extension Center in 2024.

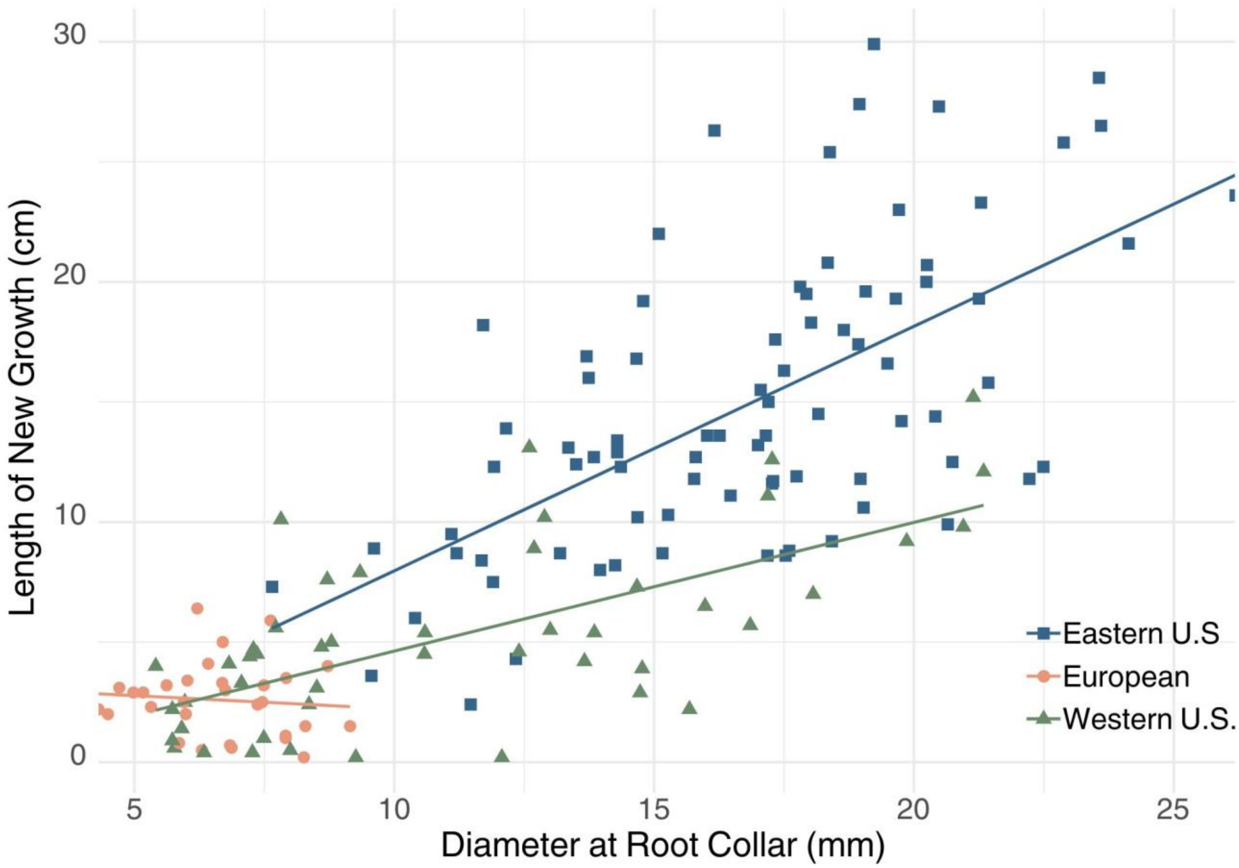

Figure 16. Length of new growth (cm) as a function of the diameter at root collar (mm) colored by species group, quantified from the modified passive approach in which seedlings were placed under BWA-infested Balsam fir at the Washington State University Puyallup Research and Extension Center in 2024.

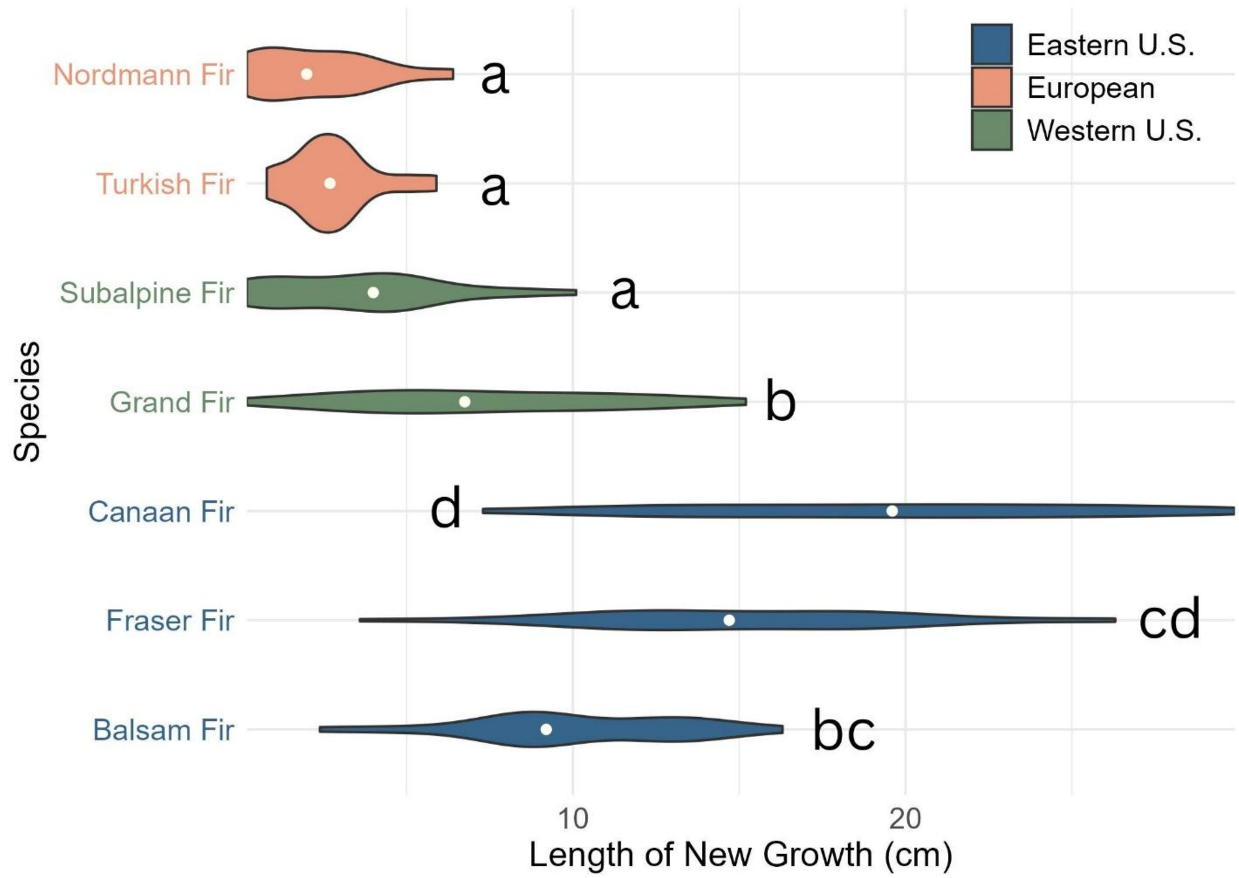

Figure 17. Length of new growth (cm) by species quantified from the modified passive approach in which seedlings were placed under BWA-infested Balsam fir at the Washington State University Puyallup Research and Extension Center in 2024. Different letters denote significant differences (α = 0.05).

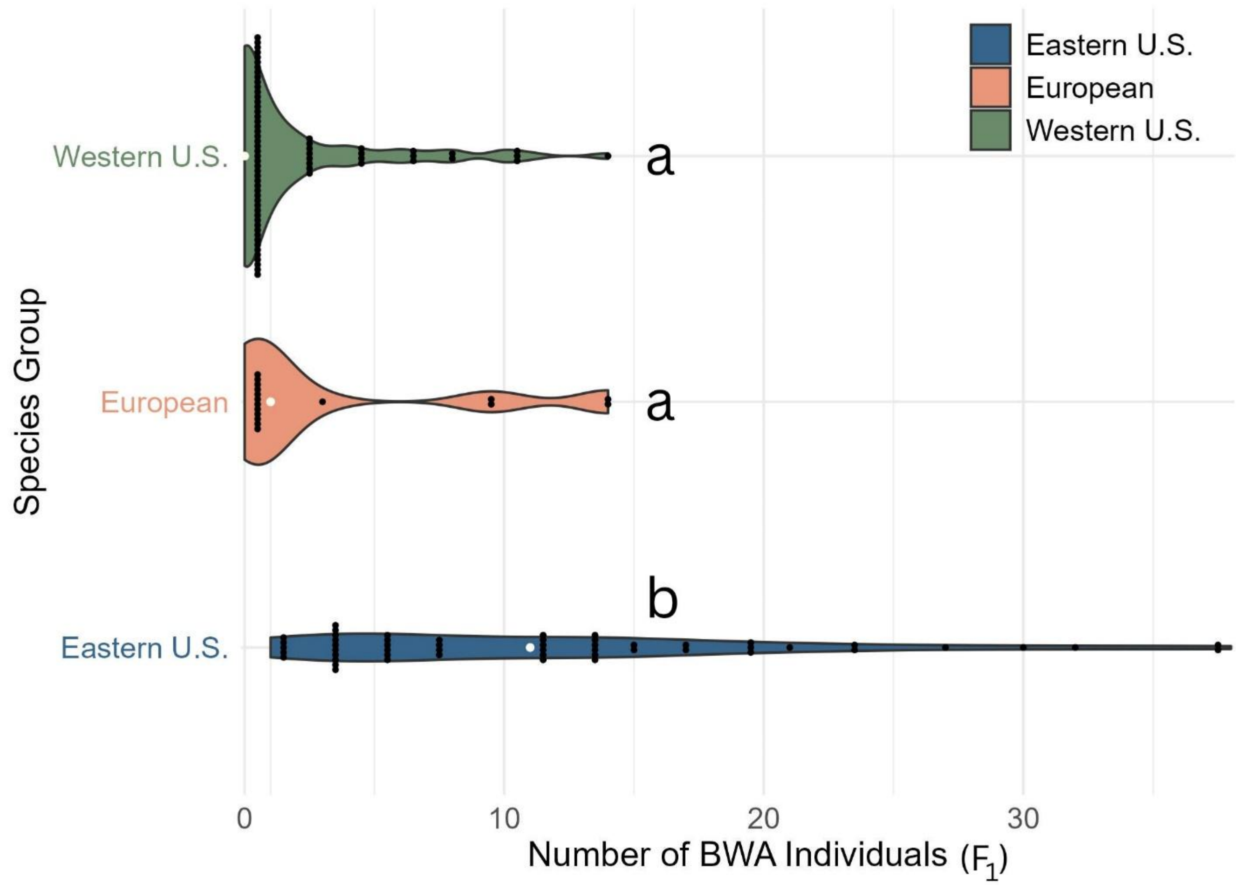

Figure 18. Number of F1 BWA individuals by species group as measured in 5-minute timed inspections. Different letters denote significant differences (α = 0.05).

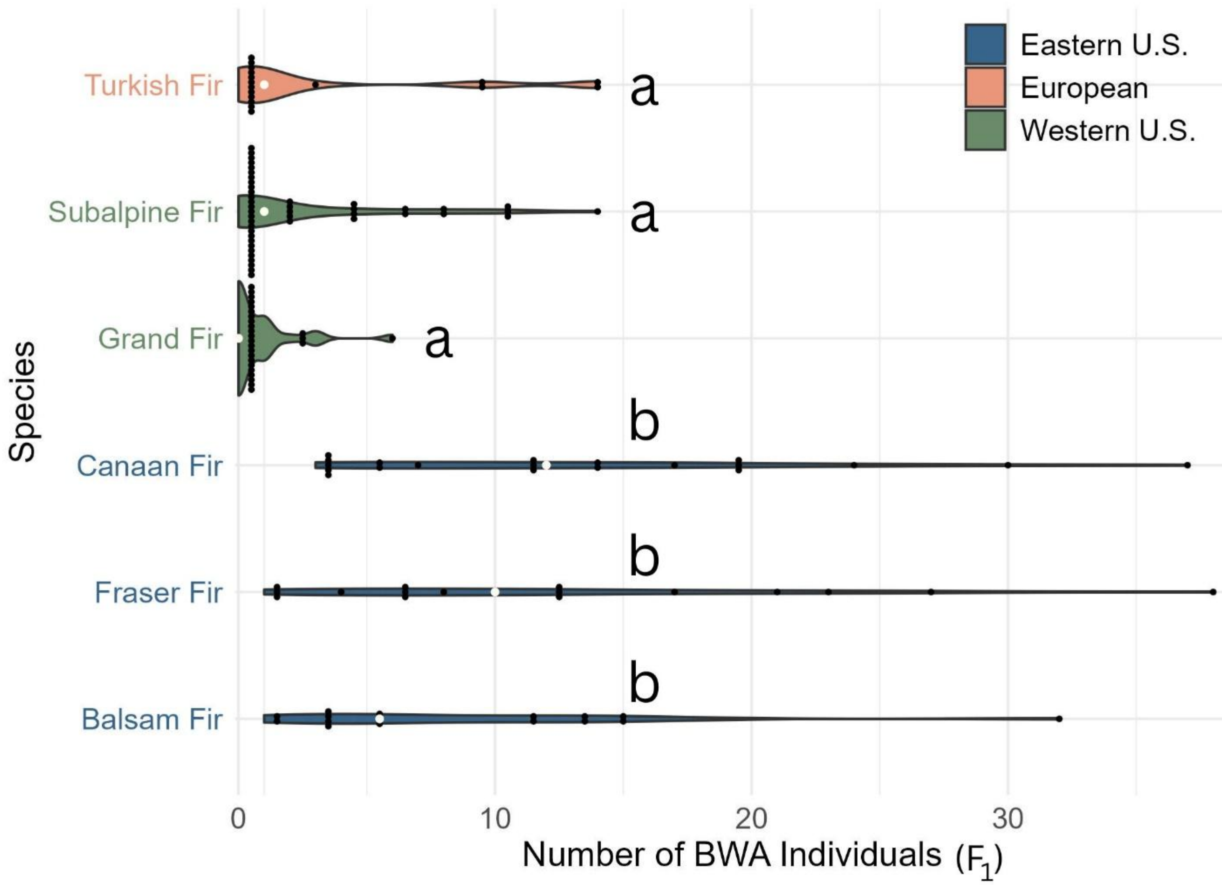

Figure 19. Number of F1 BWA individuals by species as measured in 5-minute timed inspections. Different letters denote significant differences (α = 0.05).

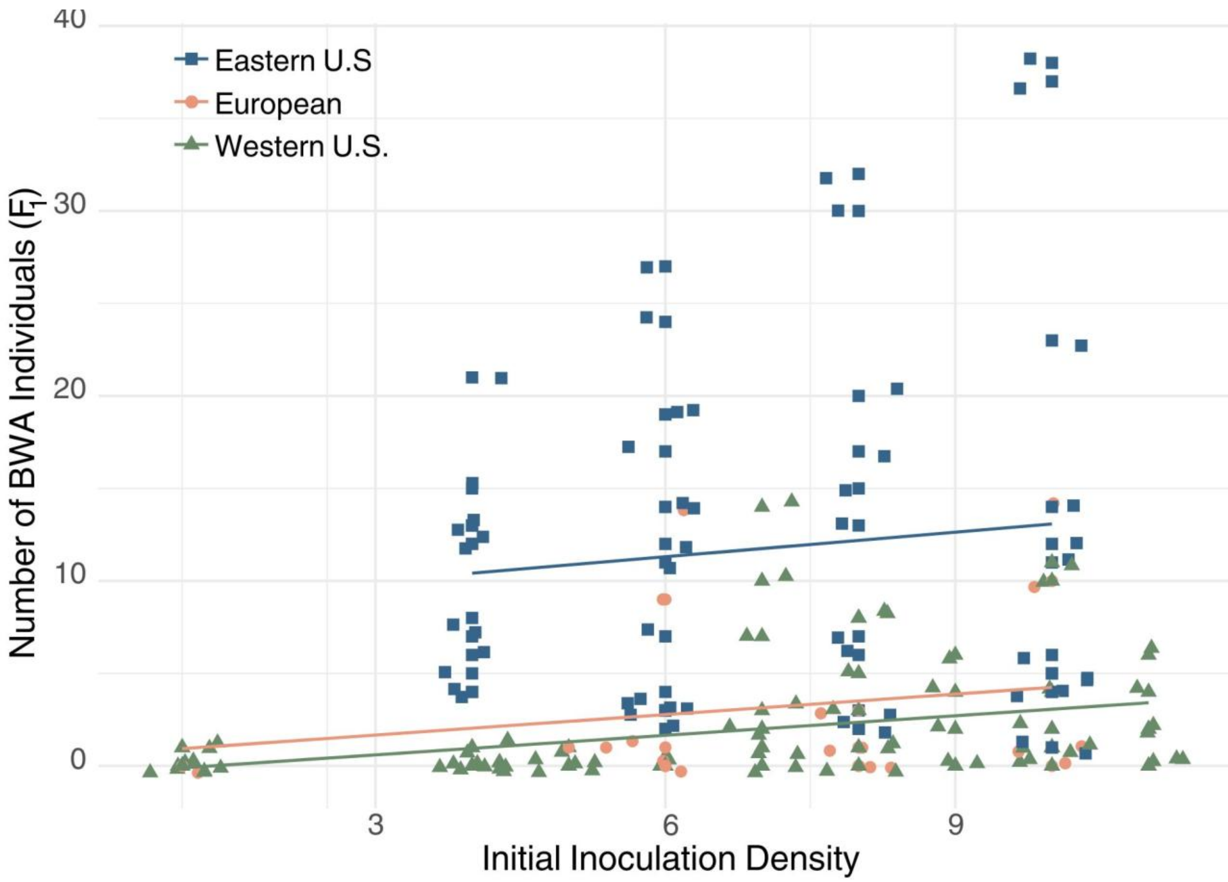

Figure 20. Number of F1 BWA individuals by initial BWA inoculation density by species group for seedlings processed using timed inspections.

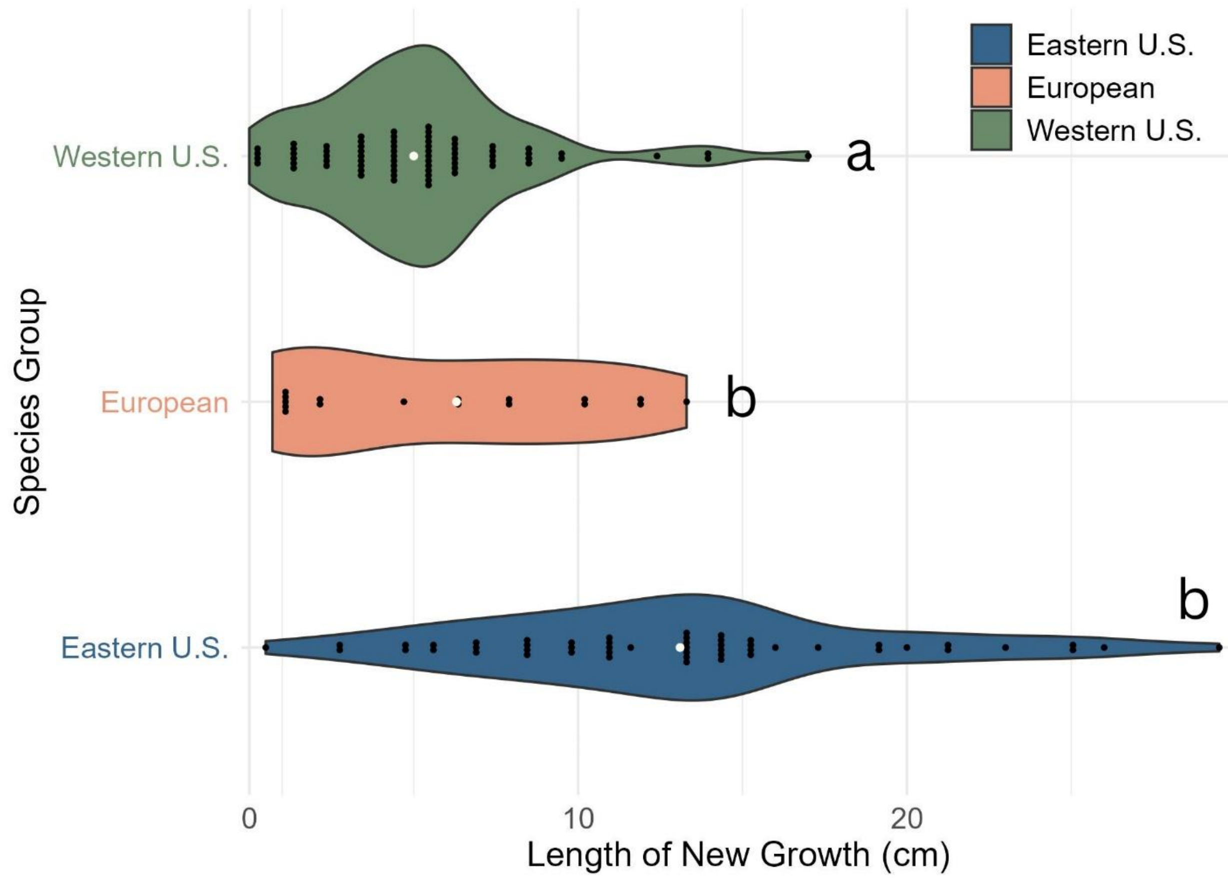

Figure 21. Length of new growth by species group from the subset of seedlings subject to a 5-minute timed inspection. Different letters denote significant differences (α = 0.05).

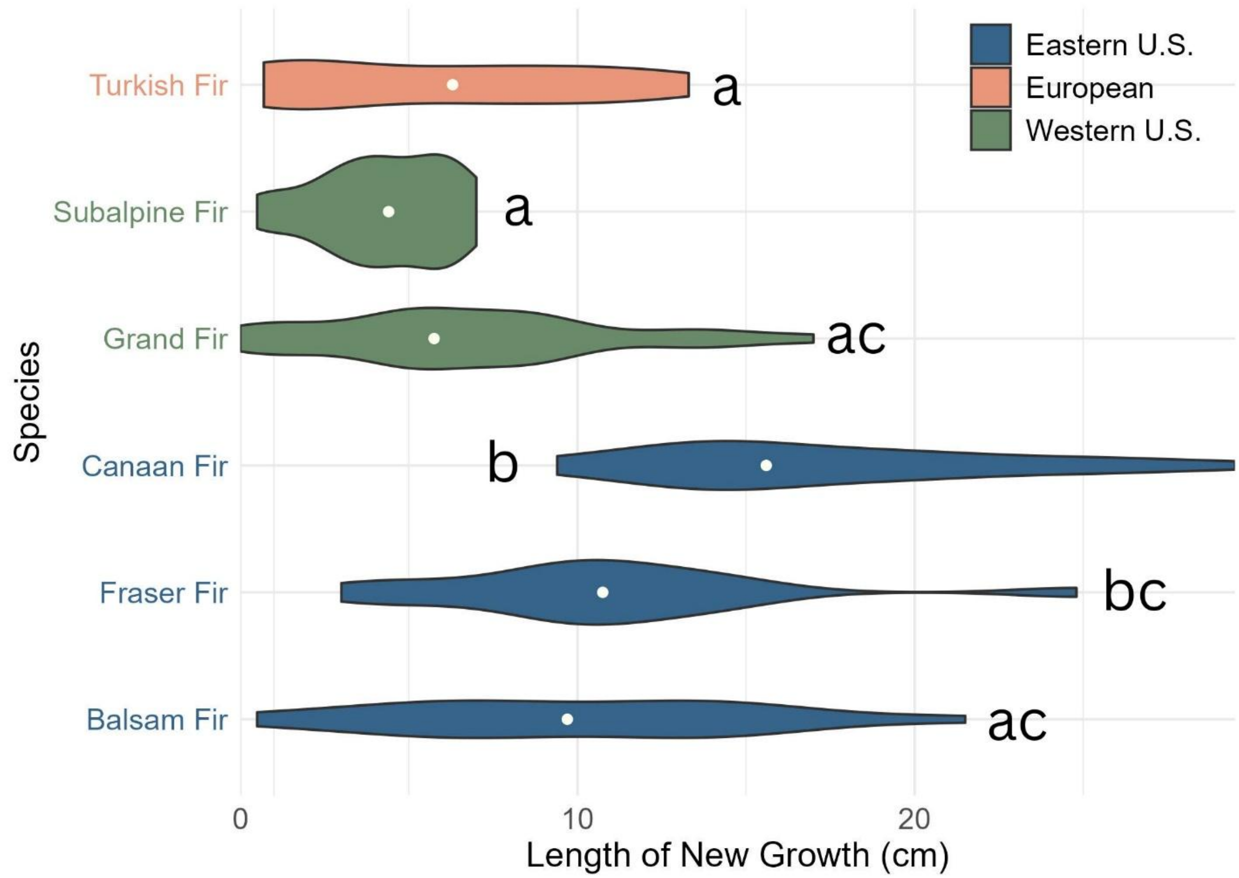

Figure 22. Length of new growth by species from the subset of seedlings subject to a 5-minute timed inspection. Different letters denote significant differences (α = 0.05).

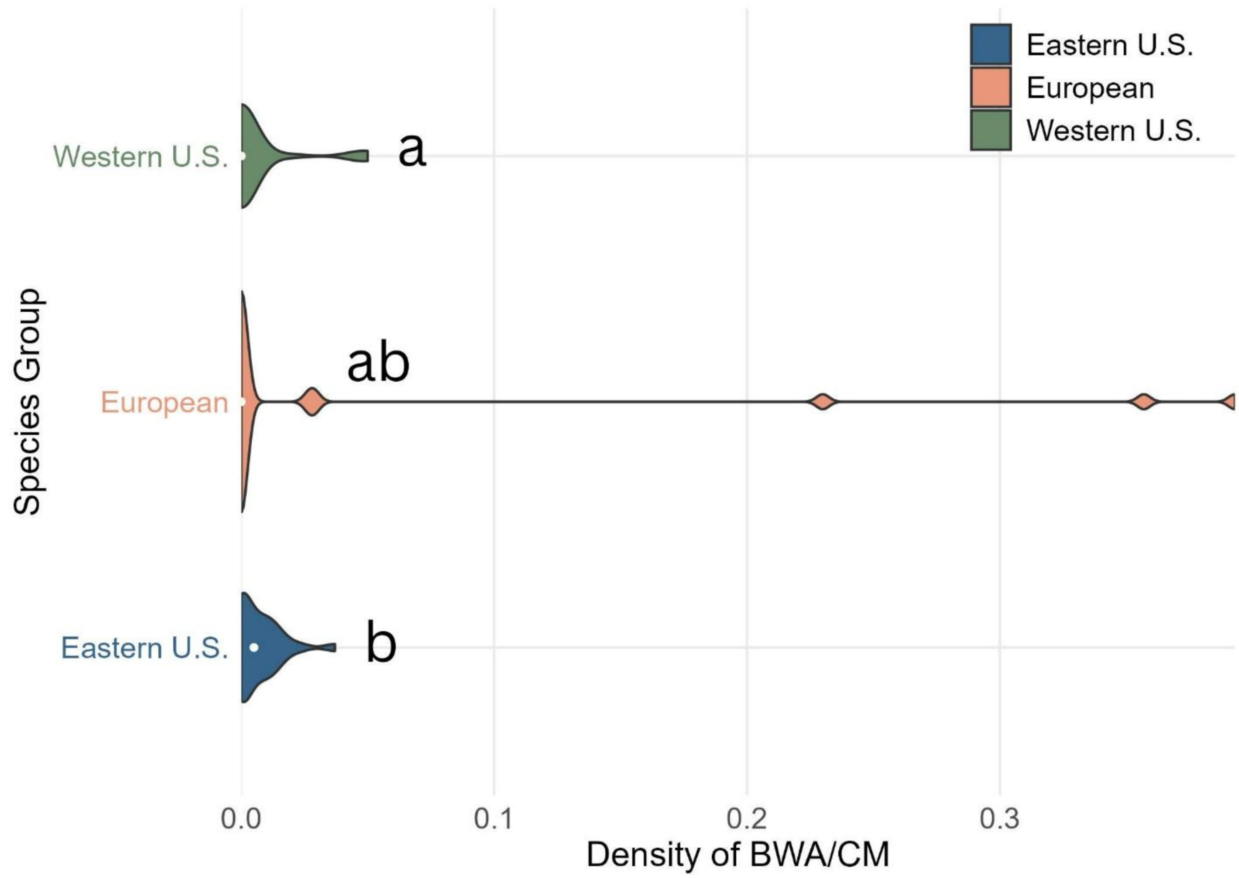

Figure 23. Density of F1 BWA individuals per cm of branch by species group from the subset of seedlings inspected without a time limit. Different letters denote significant differences (α = 0.05).

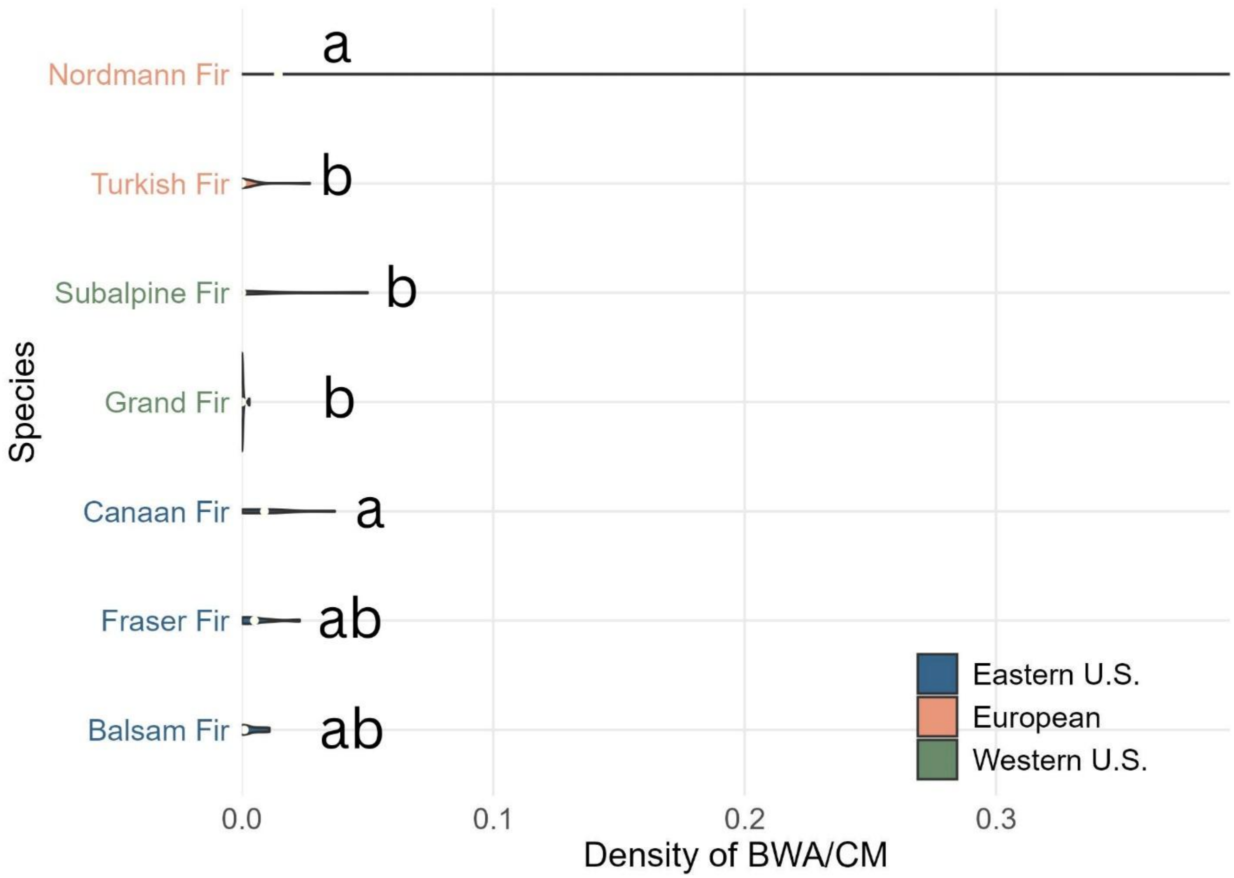

Figure 24. Density of F1 BWA individuals per cm of branch by species from the subset of seedlings inspected without a time limit. Different letters denote significant differences (α = 0.05).

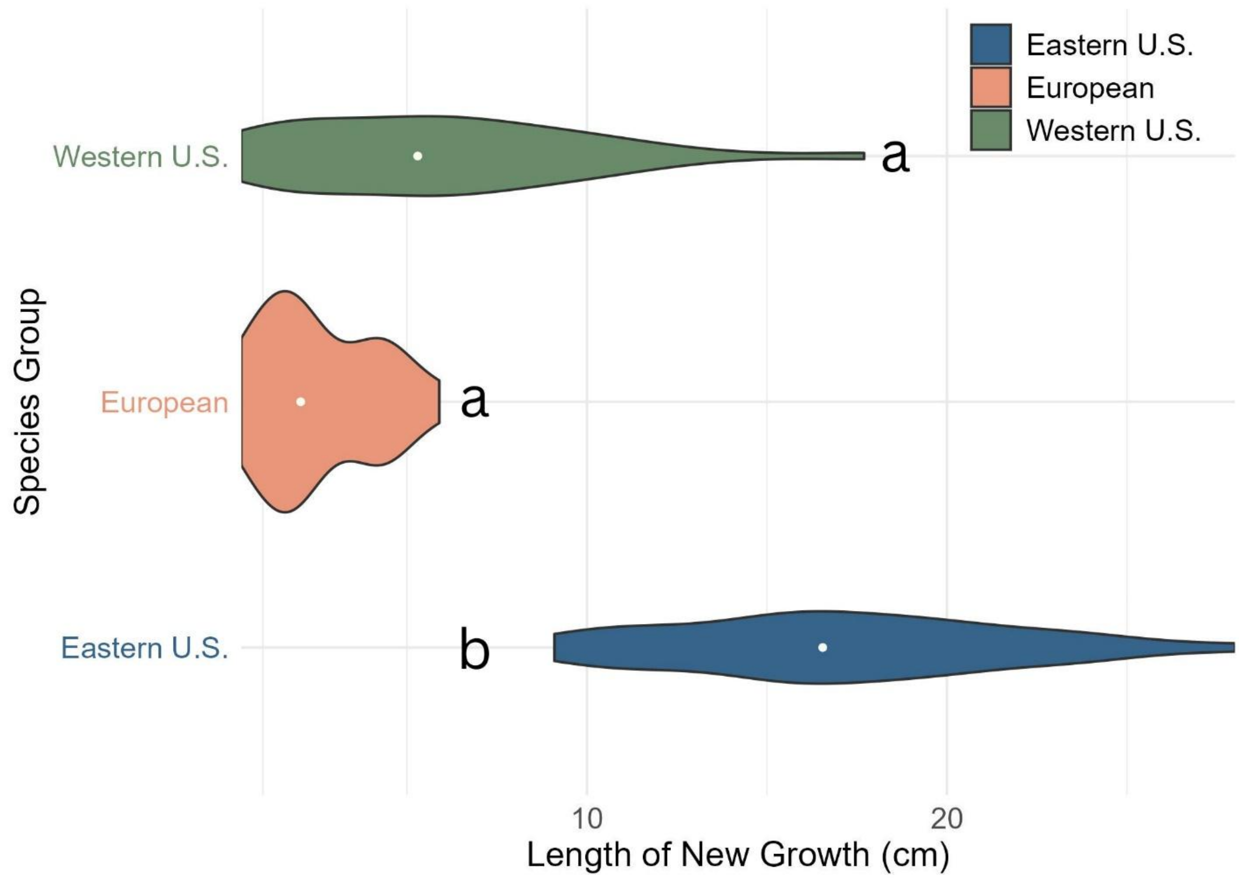

Figure 25. Length of new growth by species group from the subset of seedlings inspected without a time limit. Different letters denote significant differences (α = 0.05).

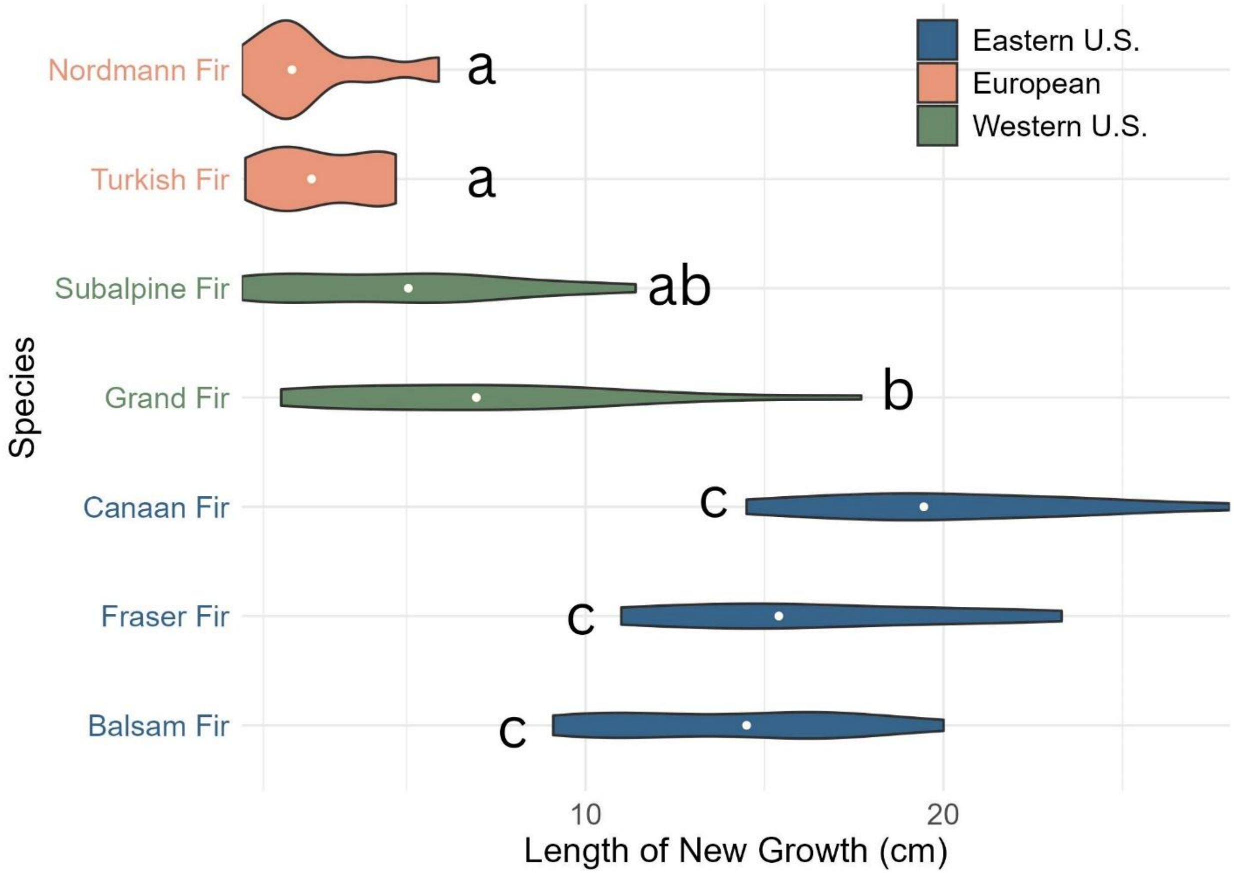

Figure 26. Length of new growth by species from the subset of seedlings inspected without a time limit. Different letters denote significant differences (α = 0.05).

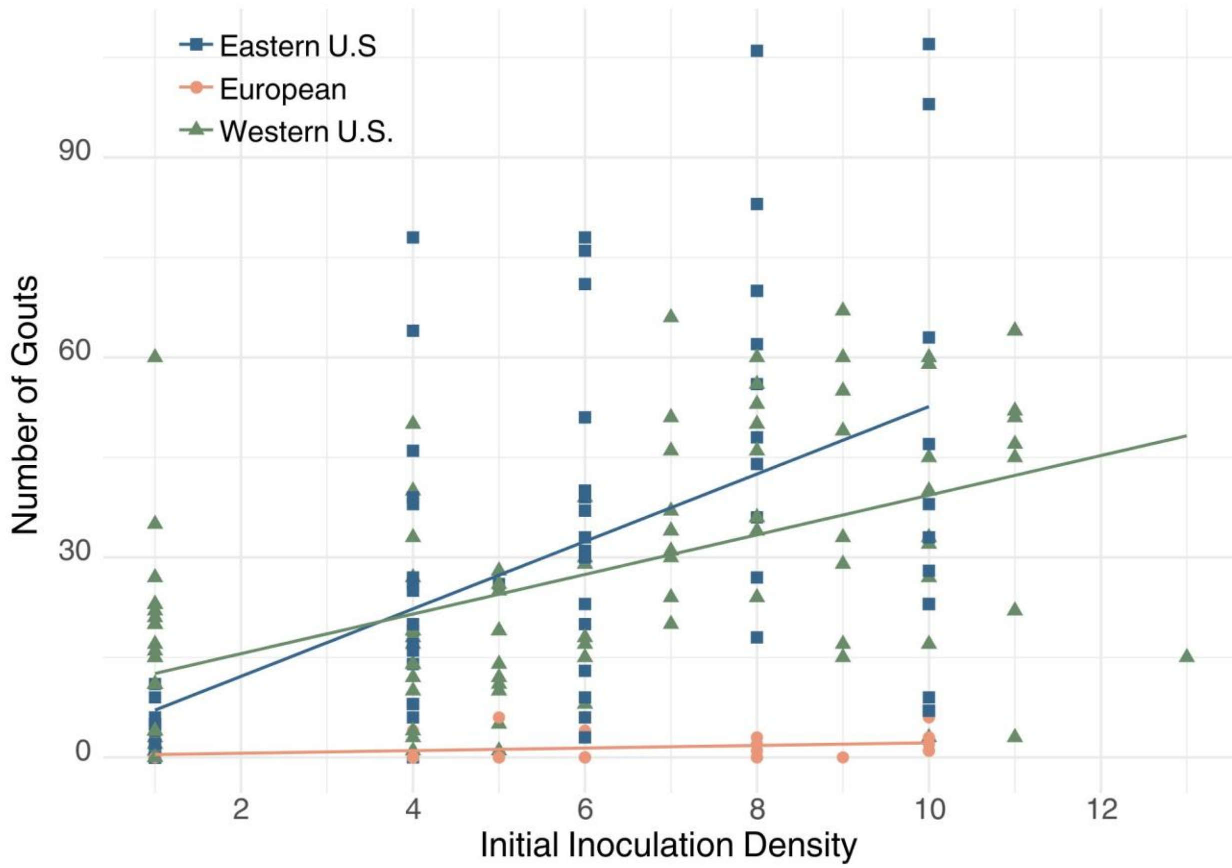

Figure 27. Number of gouts by initial BWA inoculation density by species group for seedlings processed using timed and untimed inspections.

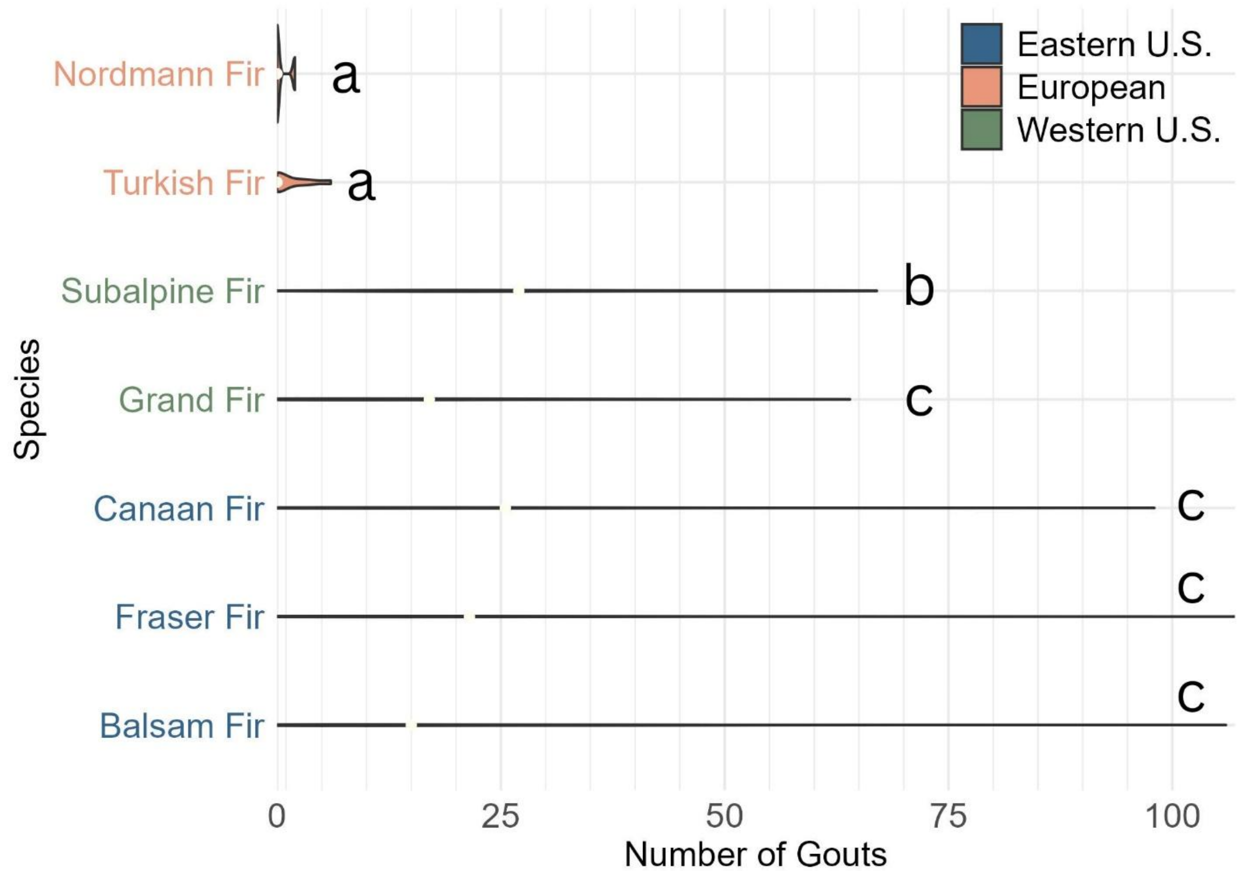

Figure 28. Number of gouts by species for seedlings processed using timed and untimed inspections. Different letters denote significant differences (α = 0.05).

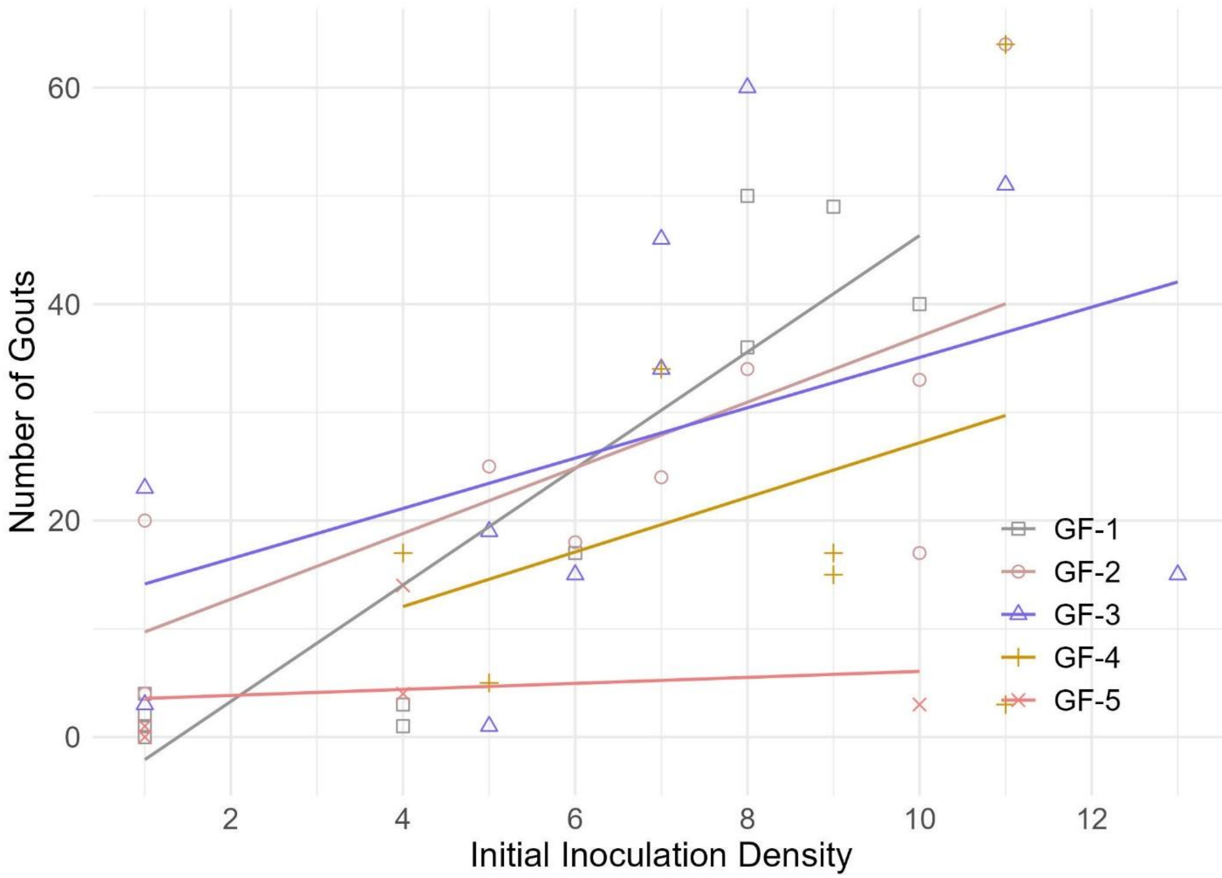

Figure 29. Number of gouts by initial BWA inoculation density by grand fir SOW number for seedlings processed using timed and untimed inspections.

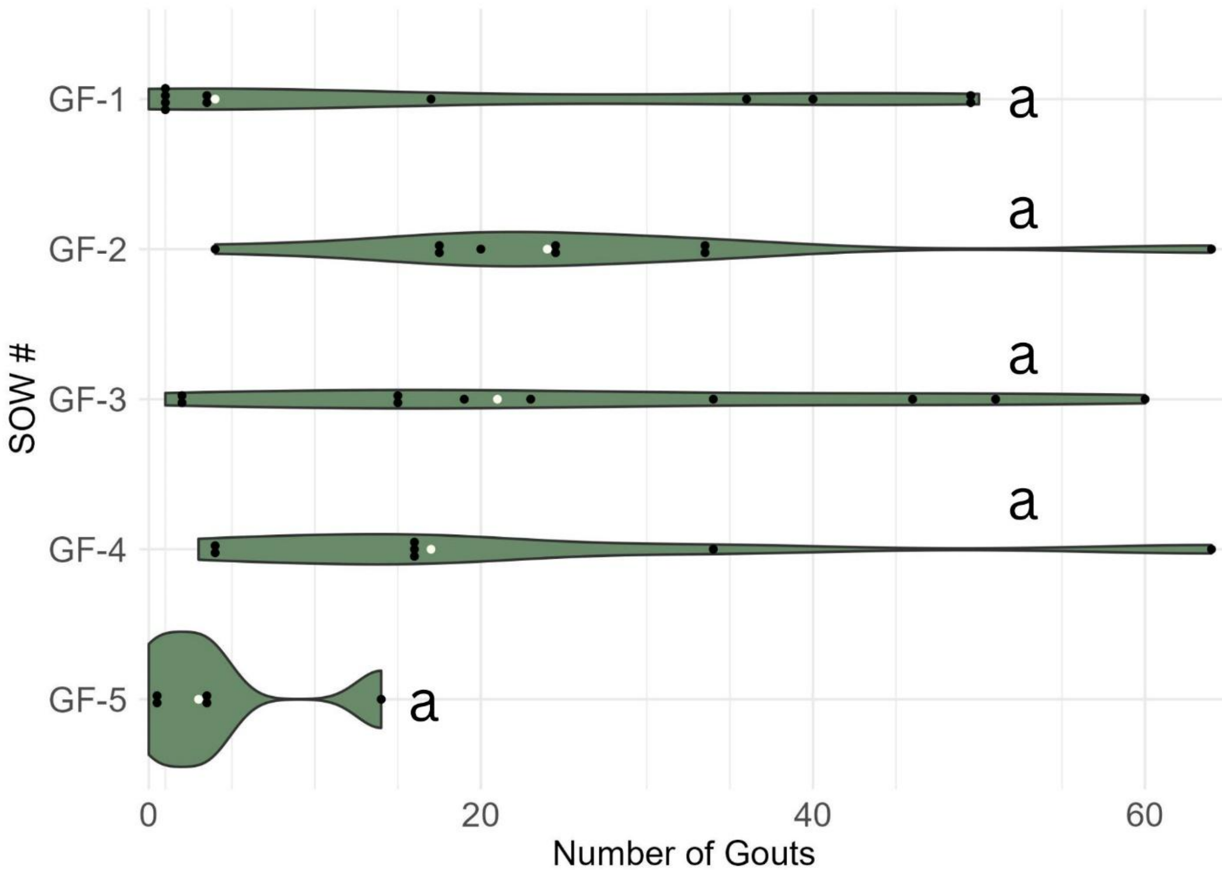

Figure 30. Number of gouts by grand fir SOW number for seedlings processed using timed and untimed inspections. The similar letters denote no significant differences (α = 0.05).

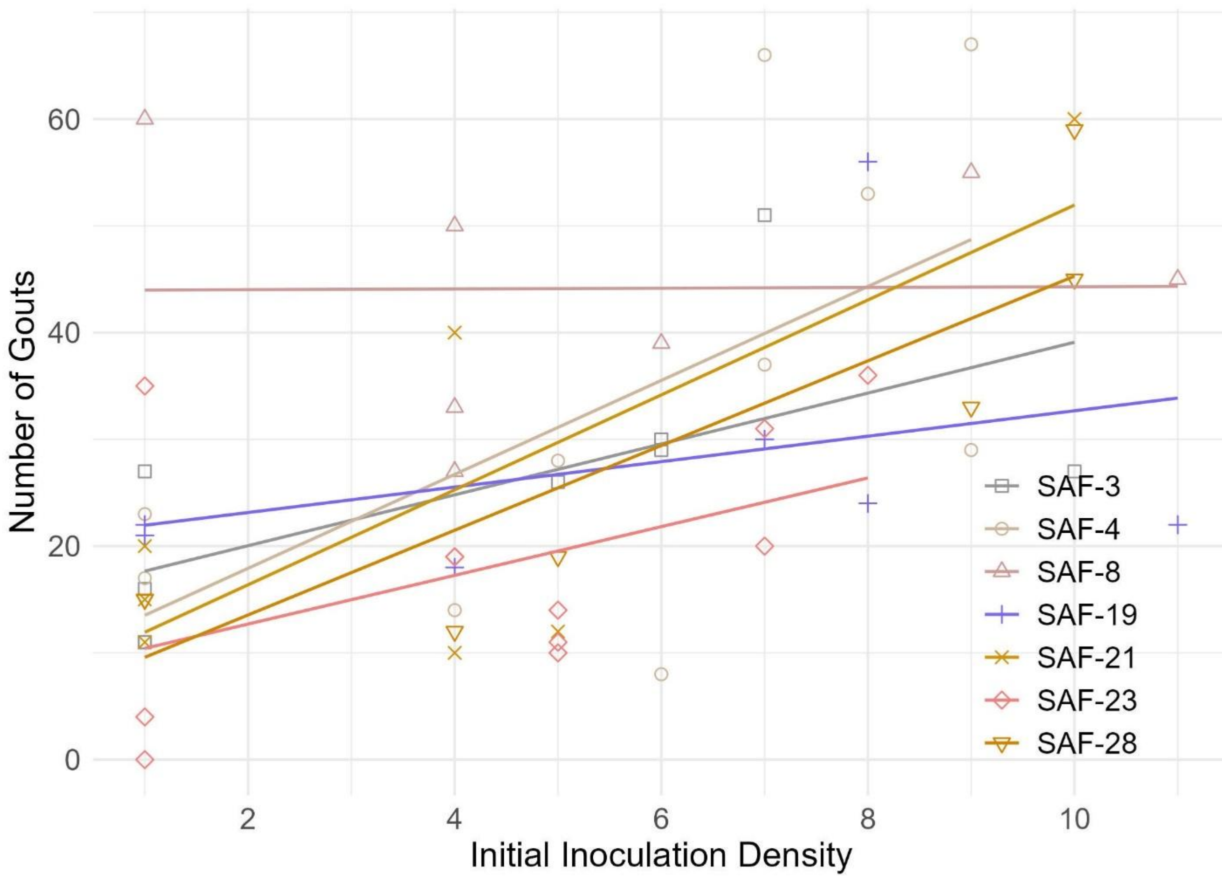

Figure 31. Number of gouts by initial BWA inoculation density by subalpine fir SOW number for seedlings processed using timed and untimed inspections.

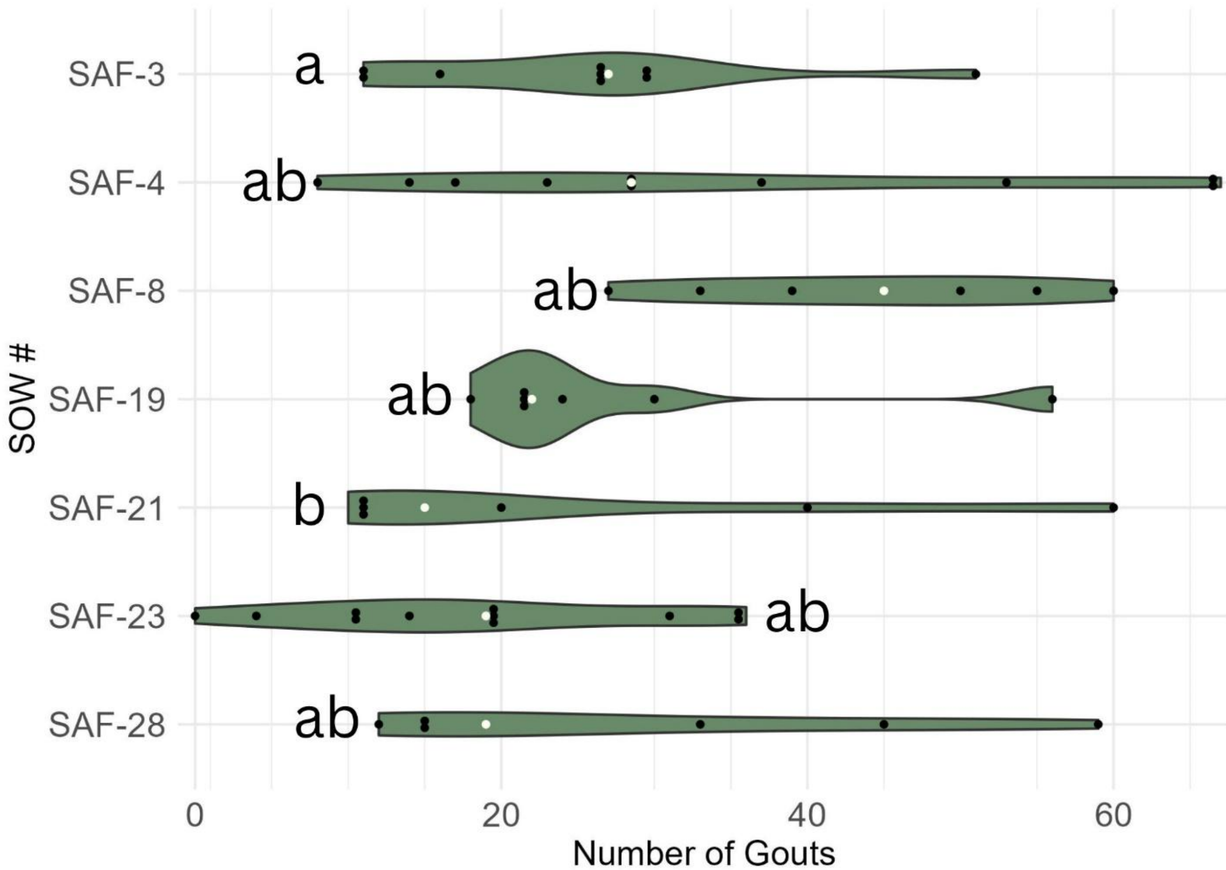

Figure 32. Number of gouts by subapline fir SOW number for seedlings processed using timed and untimed inspections. Different letters denote significant differences (α = 0.05).

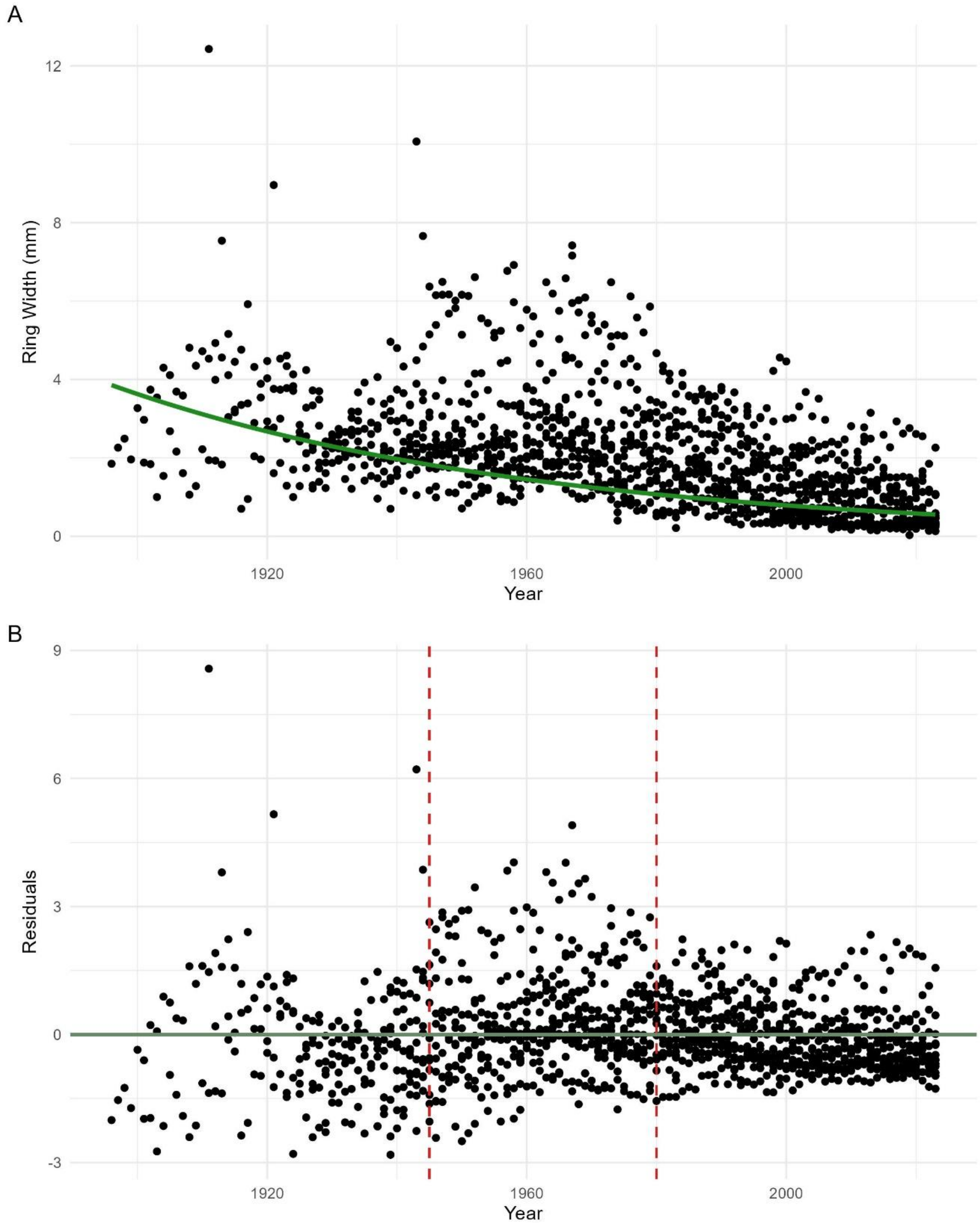

Figure 33. (A) Host tree ring widths by year from tree cores collected from the subalpine fir parent trees on the Gifford Pinchot National Forest, Washington, USA. The green line represents the predicted values from the negative exponential growth model (Eq. 1). (B) Residuals from the negative exponential mode by year, with the red dotted lines indicating the time period with greater-than-predicted tree ring widths, suggesting the possibility of Rotholz due to BWA feeding.

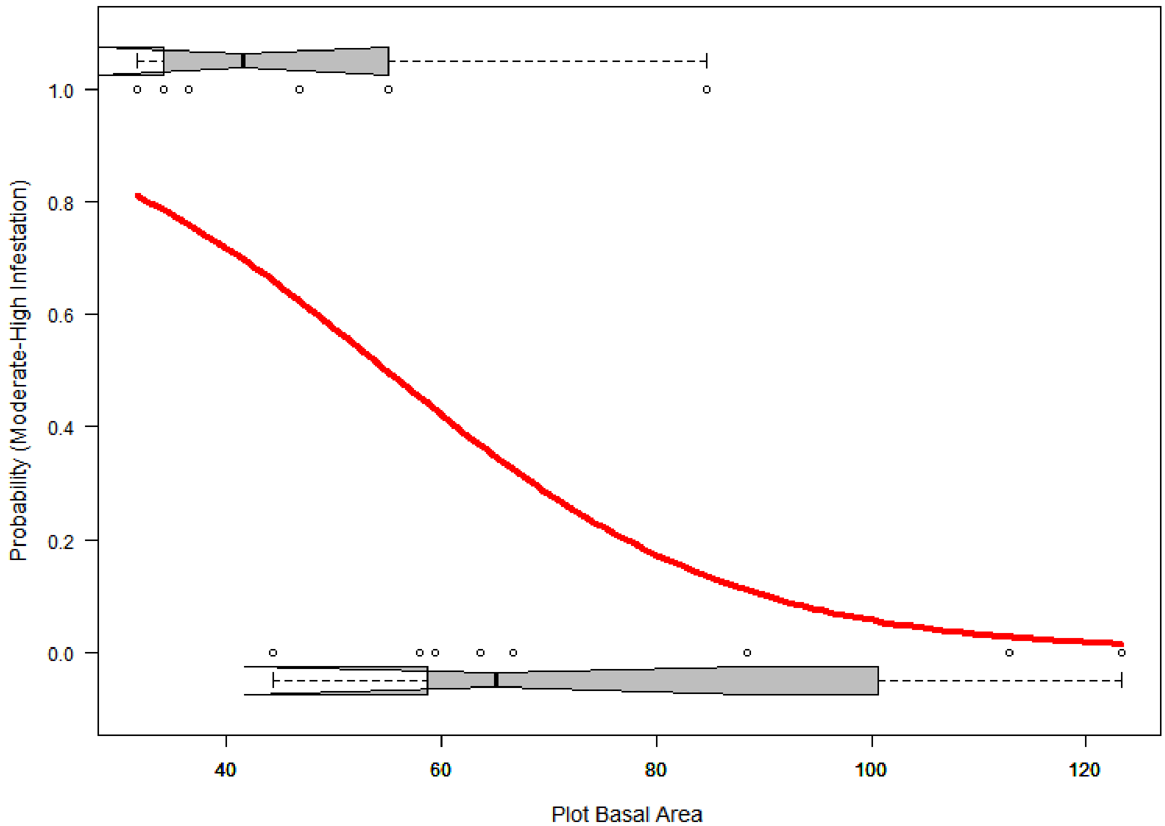

Figure 34. Predicted probability of moderate-to-high BWA infestation by plot total basal area (m2 ha-1) from the Gifford Pinchot National Forest, Washington, USA.

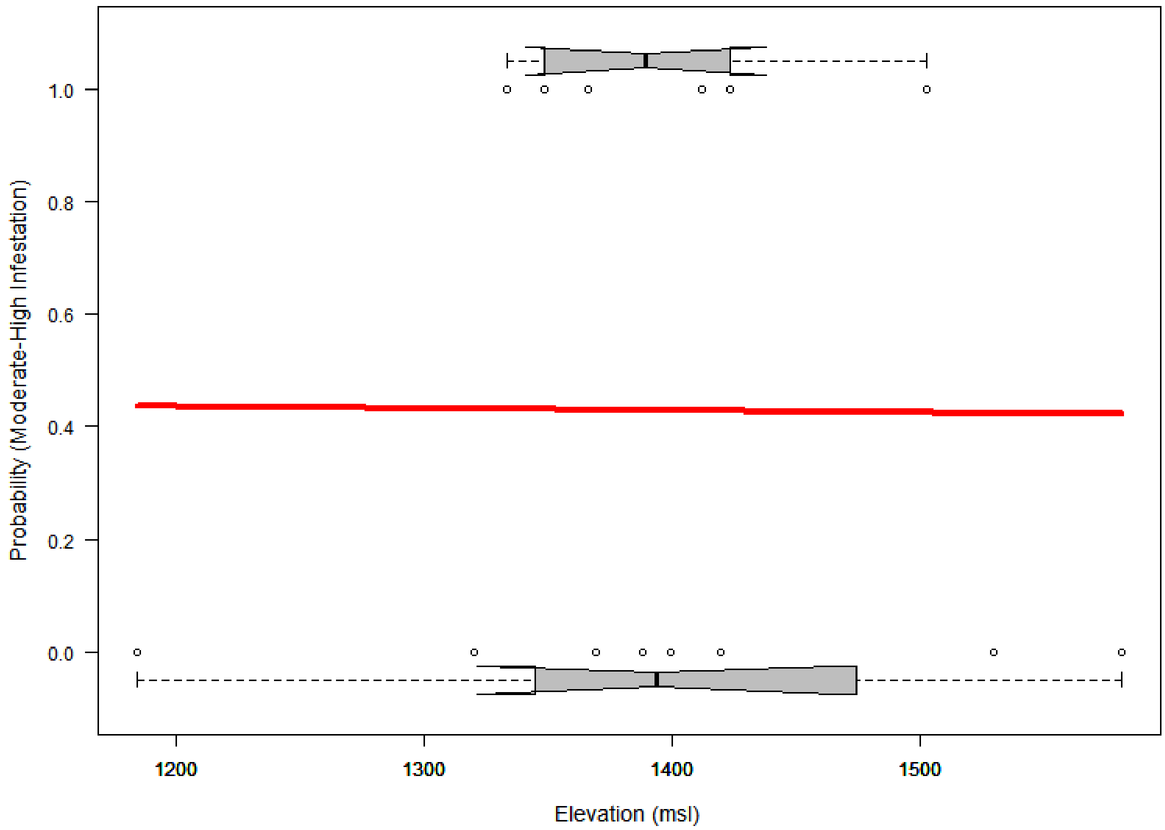

Figure 35. Predicted probability of moderate-to-high BWA infestation by the elevation of plots at the Gifford Pinchot National Forest, Washington, USA, indicating no effect of elevation on BWA infestation levels.

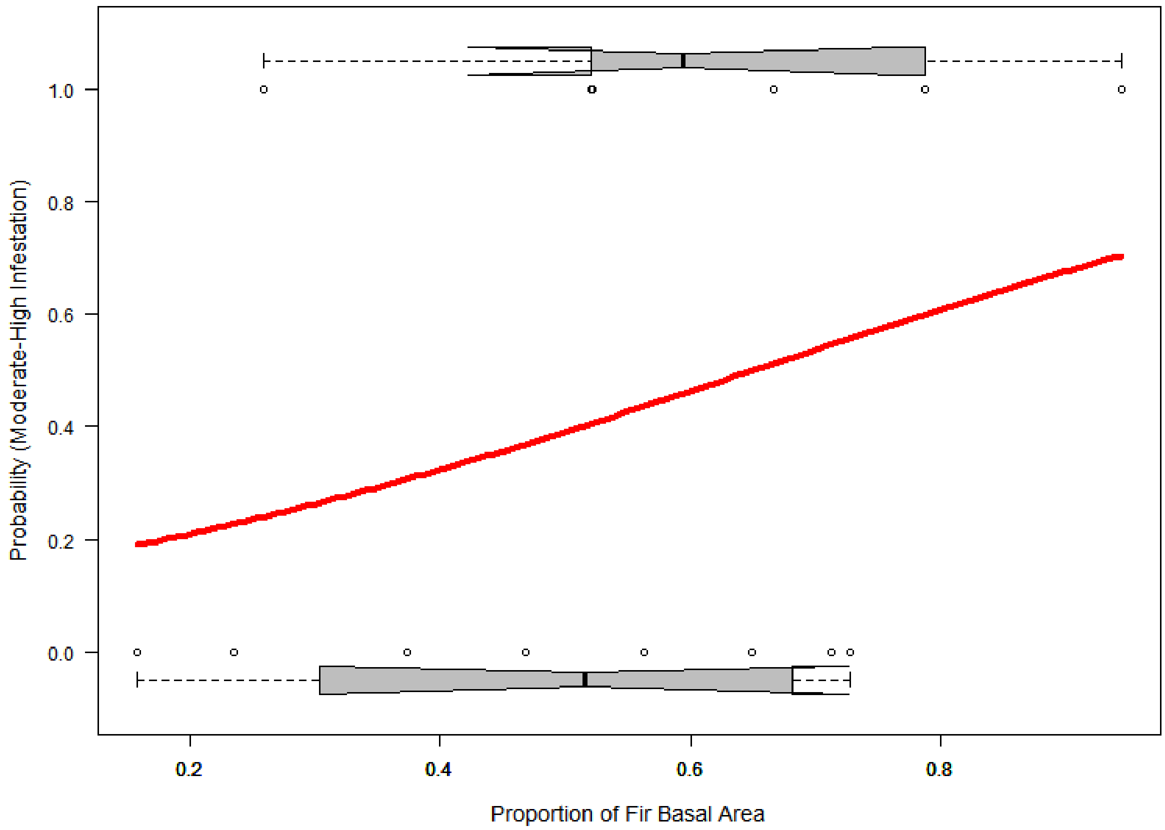

Figure 36. Predicted probability of moderate-to-high BWA infestation based on the proportion of the total plot basal area in fir from the Gifford Pinchot National Forest, Washington, USA.

Appendix

Appendix 1. Number of seedlings used for the modified passive inoculation approach and the manual inoculation approach by Species, SOW #, and Seed ID.

Species	Sow #	Seed ID	Passive	Manual Timed	Manual Untimed
Fraser Fir	FF1		32	16	10
Balsam Fir	BF1		25	16	10
Canaan Fir	CF1		25	20	13
Nordmann Fir	NF1		12		8
Turkish Fir	TF1		18	8	12
Turkish Fir	TF2			10	
Grand Fir	GF1	145106	3	6	5
Grand Fir	GF2	145006	6	7	2
Grand Fir	GF3	34418	6	6	4
Grand Fir	GF4	34403	4	3	4
Grand Fir	GF5	34003	3	4	1
Subapline Fir	SAF-3	34660	3	7	2
Subapline Fir	SAF-4	34707	3	7	3
Subapline Fir	SAF-8	34702	3	5	2
Subapline Fir	SAF-9	34703	3	2	
Subapline Fir	SAF-11	34705		1	
Subapline Fir	SAF-19	34716	4	4	3
Subapline Fir	SAF-21	35751	2	3	4
Subapline Fir	SAF-23	34767	3	7	4
Subapline Fir	SAF-24	34755	1		
Subapline Fir	SAF-28	34759	3	7	
Subapline Fir	SAF-29	34763		1	
Subapline Fir	SAF-30	34761		1	