

Article

Whitebark Pine in the Northern Cascades: Tracking the Effects of Blister Rust on Population Health in North Cascades National Park Service Complex and Mount Rainier National Park

Regina M. Rochefort ^{1,*}, Shay Howlin ², Lacey Jeroue ², John R. Boetsch ³ and Lise P. Grace ⁴

- ¹ North Cascades National Park Service Complex, 810 State Route 20, Sedro-Woolley, WA 98284, USA
- ² Western Ecosystems Technology, Inc., Environmental & Statistical Consultants, 200 S. Second Street, Laramie, WY 82090, USA; showlin@west-inc.com (S.H.); ljeroue@west-inc.com (L.J.)
- ³ National Park Service—North Coast & Cascades Network, Olympic National Park, 600 East Park Avenue, Port Angeles, WA 98362, USA; john_boetsch@nps.gov
- ⁴ National Park Service—North Coast & Cascades Network, North Cascades National Park Service Complex, 810 State Route 20, Sedro-Woolley, WA 98284, USA; lise_grace@nps.gov
- * Correspondence: regina.m.rochefort@gmail.com; Tel.: +1-360-854-7202

Received: 23 February 2018; Accepted: 26 April 2018; Published: 3 May 2018



Abstract: Whitebark pine (*Pinus albicaulis* Engelm.) is a key component of subalpine and alpine ecosystems in the northern Cascades. The species' survival is threatened by white pine blister rust, mountain pine beetle, fire exclusion, and climate change. We monitored whitebark pine in permanent plots in two national parks three times between 2004 and 2016. The proportion of live trees showing evidence of blister rust infection increased in North Cascades National Park Service Complex from 32% in 2004 to 51% in 2016 and from 18% to 38% in Mount Rainier National Park. Mortality increased from 7% to 21% in North Cascades National Park Service Complex and 38% to 44% in Mount Rainier National Park. The percent of live infected and dead whitebark pine increased with south and east aspects and mortality decreased with elevation. Annualized mortality rates calculated for the entire study period were 1.5% in Mount Rainier National Park and 2.3% in North Cascades National Park Service Complex. Although these rates decreased between the first time period (2004–2009) and the second time period (2009–2016), the prevalence of infected and dead whitebark pine increased across all park landscapes over time and increased in smaller diameter whitebark pine trees.

Keywords: *Pinus albicaulis*; whitebark pine; blister rust; national park; subalpine; Cascades; mountain pine beetle

1. Introduction

Whitebark pine is a keystone species in high-elevation areas of western North America due to its influence on biodiversity, ecosystem structure, and hydrologic cycles [1,2]. The species' survival is threatened by an introduced pathogen (*Cronartium ribicola* J. C. Fisch) that was first documented in western North America in 1910 [3,4]. *C. ribicola* is a fungus that can infect all five-needled pines, causing the white pine blister rust disease (WPBR). The fungus requires an alternate host to complete its life cycle including several species of *Ribes, Castilleja*, and *Pedicularis*, which are all widely distributed in the subalpine ecosystems [5]. Over the last 100 years, precipitous declines and widespread mortality have been documented in whitebark pine across the western United States and western Canada. In addition to WPBR, other factors implicated in the species' decline include changing fire regimes, mountain pine beetle (*Dendroctonus ponderosae* Hopkins), and warming climates [6–10]. Currently, whitebark pine is



listed as a candidate for protection under the U.S. Federal Endangered Species Act and as endangered under the Species at Risk Act (SARA) in Canada [11,12].

Historical accounts of the spread of WPBR from areas near North Cascades National Park Service Complex are limited to one 1913 report of a potential pine infection near Newhalem, Washington on the west edge of the park [4]. In contrast, Mount Rainier National Park was a center of activity from the late 1920s through the 1960s. Despite widespread *Ribes* eradication programs, 52–55% of whitebark pines in the Sunrise area (Figure 1) were recorded as infected in 1952. In the late 1950s and 1960s, work in Mount Rainier National Park turned to treatment with fungicides and surveys for western white pine that appeared to have genetic resistant to WPBR. Although the scenic value of whitebark pine was repeatedly mentioned in park memos, efforts to control WPBR through fungicides and *Ribes* removal were terminated around 1964 when it was determined neither tactic was effective in reducing the spread of WPBR [13,14].

Between 1994 and 1999, national park staff conducted inventories in both parks to quantify the incidence of WPBR and mountain pine beetle in whitebark pine stands [15]. Surveys revealed that about one third of all trees surveyed were dead, incidence of WPBR infection was highly variable (0–70%), and observations of mountain pine beetle were rare. Contemporaneous studies conducted in the Washington and Oregon Cascades documented similar patterns: high variability among sites, limited mountain pine beetle activity, WPBR infection of live trees ranging from 4.7–73%, and mortality ranging from 10–23% [8,16,17]. All studies noted difficulty in attributing the cause of mortality since many trees had been dead for many years.

Following initial inventories, we realized that long-term monitoring plots with marked trees were needed to attribute the cause of mortality and to track long-term trends in population health [6,7,10,15,16,18]. In 2004, we established permanent plots in each park to document status and trends in whitebark pine population health and to provide a foundation of scientific data to inform national park conservation strategies and management tactics. Specifically, our objectives were: (1) describe the current composition and structure of whitebark pine stands; (2) quantify status and trends in incidence of WPBR infection and mortality in trees and saplings; (3) quantify the incidence of mountain pine beetle; (4) describe status and trends in seedling density; and (5) evaluate landscape patterns of WPBR infection and whitebark pine mortality.

2. Materials and Methods

2.1. Study Area

The study was conducted in the subalpine parklands of North Cascades National Park Service Complex and Mount Rainier National Park in the northern Cascade Mountains of Washington State (Figure 1). North Cascades National Park Service Complex encompasses three areas administered by the National Park Service: North Cascades National Park, Ross Lake National Recreation Area, and Lake Chelan National Recreation Area. North Cascades National Park Service Complex extends from the Canadian border southeast along the Cascade Crest for about 82 kilometers (km), encompassing 276,815 hectares (ha) with an elevation range of 199–2806 m. North Cascades National Park Service Complex spans two very different biogeographic zones. The west slopes of the Cascades experience a wet, temperate maritime climate with annual precipitation of up to 897 centimeters (cm), while the drier eastern slopes have a more continental climate with annual precipitation of about 76 cm. Mount Rainier National Park encompasses 95,781 ha from low-elevation old-growth forests at 490 m in elevation to the summit of Mount Rainier at 4392 m. Whitebark pine is distributed in scattered stands above 1800 m in the northeast corner of Mount Rainier National Park and in the southeastern portion of North Cascades National Park Service Complex.





Figure 1. Study sites and whitebark pine distribution in (**a**) Mount Rainier National Park and (**b**) North Cascades National Park Service Complex. Study sites are labeled and indicated by black points.

2.2. Field Methods

We used a two-stage sample design with whitebark pine sites as the primary sampling units and circular 0.4 ha plots as the secondary units, sampled from within the primary units. Eight whitebark pine sites in Mount Rainier National Park and five in North Cascades National Park Service Complex were selected. Using inventory data, we calculated the number of plots needed per study site to estimate the proportion of dead trees and proportion of WPBR infected trees with precision in the range of 0.1–0.2 [15,19]. Study sites and plots were randomly selected from whitebark pine distribution maps assembled from historic maps and more recent vegetation surveys [15,18–21]. These maps delineated 66 whitebark stands in Mount Rainier National Park totaling 1433 ha and 12 stands in North Cascades National Park Service Complex encompassing 923 ha.

Plots were randomly located in each study site in 2004 and then resurveyed in 2009 and 2015–2016. Two sites in North Cascades National Park Service Complex were surveyed in 2016 because we could not complete the 2015 surveys before snowfall (Table 1). Plot centers were marked with rebar, locations recorded with a global positioning system unit (GPS), and slope, aspect, and elevation were recorded. Within each plot, all trees greater than or equal to 2.54 cm diameter at breast height (dbh, 1.37 m), living and dead, were identified to species; dbh, height, and presence or absence of fire scars were recorded. All whitebark pine trees within the plot were tagged with aluminum tags and additional data were recorded: presence or absence of cones, presence of cankers, animal gnawing on branches, and evidence of pine beetle. When cankers were present, we recorded whether they were located on branches or the tree bole and whether they were active or inactive [22]. We considered presence of any cankers or animal gnawing as indicators of WPBR infection [22]. We examined whitebark pine trees for evidence of mountain pine beetle and recorded beetles as present if we found pitch tubes, exit holes, or visible galleries on dead trees after bark had fallen off. When whitebark pine trees were growing in a clump, we followed the stem to the ground to determine if stems were individual trees or multi-stemmed individuals following a prescribed methodology [19]. All saplings greater than or equal to 50 cm in height and less than 2.54 cm dbh and seedlings less than 50 cm in height were identified to species and tallied. Whitebark pine saplings were examined for presence of cankers and if present, recorded as infected with WPBR.

Site	Mean Elevation — (meters)	Number of Plots Sampled		
		2004	2009	2015/16 ¹
Mount Rainier National	Park			
Antler Peak	1858	4	4	4
Crystal Ridge	1927	5	5	5
Frying Pan	1817	3	3	3
Glacier Basin	2033	5	5	5
Mystic Lake	1872	3	3	3
Skyscraper	1962	2	2	2
Sunrise Campground	1940	5	5	5
Palisades	1883	2	2	2
North Cascades Nationa	l Park Service Comp	olex		
Dee Dee Lake	1976	7	7	7
Juanita Lake	2084	7	7	7
Rainbow Lakes	1870	7	7	7
Rainbow Ridge	1987	7	7	7
Stiletto	1938	7	7	7

Table 1. Sample sites (primary sample units) and plots (secondary sample units) established at Mount Rainier National Park and North Cascades National Park Service Complex.

¹ Rainbow Lakes and Stiletto were sampled in 2016.

2.3. Data Analysis

2.3.1. Site Composition and Structure

Community composition of trees, saplings, and seedlings was examined for each study site by comparing basal area of all tree species and densities of trees, saplings, and seedlings. Basal area and densities were averaged across all plots within a site.

2.3.2. Population Health of Whitebark Pine Trees, Saplings, and Seedlings

We described whitebark pine population health by calculating the mean percent of trees that were dead, live infected with WPBR, and live uninfected with WPBR at each time period. To provide inference to the entire whitebark pine population for each park, percentages for each park were constructed using means for each site weighted by the size of the site [23]. All analyses were conducted using R version 3.4.2 [24].

Population health was examined in two ways: cumulatively and progressively. Cumulative estimates of population health included all dead trees documented during each of the three monitoring periods. Progressive analyses removed previously dead trees from subsequent analysis (e.g., if a tree was recorded as dead in 2004, it was removed from the data set for 2009 and 2015). Our reason for doing this was twofold: first, cumulative analysis describes the image at one point in time because standing dead trees can persist for years. Progressive analysis provides insight into the temporal movement of WPBR incidence and mortality through the study sites by examining changes in mortality incidence across the monitoring time period. In progressive analyses, we removed one Mount Rainier National Park site (Frying Pan) from our analysis because two of the three plots had no live trees remaining after 2004, and we could not obtain site level averages.

To compare trends in mortality between parks and time periods, we annualized mortality rates for three time periods: 2004 to 2009, 2009 to 2015/2016, and 2004 to 2015/2016. The number of intervening years was used to standardize the percent newly dead estimate. The averages across sites were calculated for a naïve form of annualized mortality rate.

Saplings were summarized by mean percent dead, live uninfected, and WPBR live infected; live whitebark pine were classified as infected if cankers were documented during field surveys. Live seedlings were tallied and average density calculated. Sapling and seedling averages were calculated across study sites rather than with inference to each park.

2.3.4. Landscape Patterns of Whitebark Pine Mortality, Blister Rust Infection, and Prevalence

2.3.3. Population Health and Density of Saplings and Seedlings

Landscape level patterns of whitebark pine health were summarized by WPBR infection and total mortality. Tree mortality and WPBR infection incidence were investigated using site level data and linear mixed effect models based on the normal distribution. Normality was assumed based on histograms of the data and validated based on quantile-quantile plots which compared the distribution of mean mortality and infection to a normal distribution. Repeated observations for a site across each of the three study years were accommodated by including a random site effect in the intercept. A sample unit was a site, and 13 sites across three years represented the sample size used to fit the models (n = 39). Models were fit with all combinations of the covariates elevation, slope, northness, eastness, x location, y location, dbh, and park, summarized at the site level. Northness was calculated as the cos(aspect) with values of 1 indicating north facing slopes and values of -1 indicating south facing slopes. Eastness was calculated as the sine(aspect) with values of 1 indicating east facing slopes and values of -1 indicating west facing slopes. Because northness was calculated directly from aspect, both covariates were not allowed to enter the same model to avoid multicollinearity among covariates. Models were chosen based on the corrected Akaike information criterion (AICc) for finite populations, where the smaller AICc indicated the relative ranking among candidate models. We performed a leave-one-out model validation on the best model to understand how well the model fit the data. We did this by leaving out a plot from the dataset used to fit a model, then using that model to predict the one plot left out of the dataset. We compared the predicted values with the actual values and computed a root mean square error (RMSE) and bias.

Whitebark pine population health was summarized at the landscape level in terms of prevalence of blister-rust infected whitebark pine trees on the landscape. Prevalence was defined as the proportion of infected or dead whitebark pine trees across all plots and sites. Dead trees were combined with live WPBR infected trees because, based on previous and current surveys, we concluded that most mortality of whitebark pine trees was caused by WPBR [15]. We used the cumulative data on repeated whitebark pine health measurements to fit WPBR prevalence models. The sample unit was an individual whitebark pine, and a total sample size of 1661 whitebark pine records across 2004, 2009, and 2015/2016 survey years from plots was available for model fitting. We fit a generalized linear mixed effect model with a binomial distribution and logit link incorporating a random effect of nested plot and tree in the intercept to address the lack of independence among repeated tree measurements across years and between whitebark pine trees on a single plot. Models were fit with all combinations of the covariates park, site, dbh, dbh², and year, and interactions between park and year, and dbh and year. Because park and site were highly correlated, both covariates were not allowed to enter the same model. Model forms were restricted so that both main effects were included if the interaction term was in the model. In addition, models with the quadratic term for dbh (dbh^2) also contained the linear term. Frying Pan was dropped from this analysis because dbh data were not collected for most of the whitebark pine trees in 2009. The best model was chosen based on AICc. We performed a leave-one-out model validation on the best model to understand how well the model fit the data. We did this by leaving out a plot from the dataset used to fit a model, then using that model to predict the one plot left out of the dataset. We compared the average predicted prevalence with the actual average prevalence and computed the RMSE and bias.

3. Results

3.1. Current Composition Status of Whitebark Pine Sites

We documented nine tree species in our study sites, eight in Mount Rainier National Park and six in North Cascades National Park Service Complex (Figure 2a,b). Subalpine fir (*Abies lasiocarpa*), silver fir (*Abies amabilis*), Engelmann spruce (*Picea engelmannii*), mountain hemlock (*Tsuga mertensiana*), and whitebark pine were recorded in both parks. Subalpine larch (*Larix lyallii*) grows only in North Cascades National Park Service Complex and Alaska yellow cedar (*Chamaecyparis nootkatensis*), ponderosa pine (*Pinus ponderosa*), and lodgepole pine (*Pinus contorta*) were only recorded in Mount Rainier National Park plots. In all sites except Juanita Lake, subalpine fir was the dominant species, and whitebark pine contributed less than 20% of the total basal area in all sites in Mount Rainier and in two sites in North Cascades National Park, Glacier Basin had the highest percent of whitebark pine (17.4%). In North Cascades National Park Service Complex, whitebark pine comprised 43% and subalpine larch comprised 33% of basal area in the Juanita Lake area. In 2015/2016, total basal area ranged from 9–45 square meters (m²)/ha in Mount Rainier National Park and 6-16 m²/ha in North Cascades National Park Service Complex sites.



Figure 2. Cont.



Figure 2. Site composition in 2015/2016 for trees, saplings, and seedlings. Mean tree basal area by species in (**a**) Mount Rainier National Park and (**b**) North Cascades National Park Service Complex. Mean sapling density by site in (**c**) Mount Rainier National Park and (**d**) North Cascades National Park Service Complex. Mean seedling density by site in (**e**) Mount Rainier National Park and (**f**) North Cascades National Park Service Complex. Species abbreviations are as follows: ABIAMA = silver fir (*Abies amabilis*), ABILAS = subalpine fir (*Abies lasiocarpa*), CHANOO = Alaska yellow cedar (*Chamaecyparis nootkatensis*), LARLYA = subalpine larch (*Larix lyallii*), PICENG = Engelmann spruce (*Picea engelmannii*), PINALB = whitebark pine (*Pinus albicaulis*), PINCON= lodgepole pine (*Pinus contorta*), PINPON = ponderosa pine (*Pinus ponderosa*), TSUHET = western hemlock (*Tsuga heterophylla*), and TSUMER = mountain hemlock (*Tsuga mertensiana*).

We recorded nine tree species in sapling and seedling surveys (Figure 2c–f). Ponderosa pine was not found in regeneration in either park. Western hemlock (*Tsuga heterophylla*) was recorded only in the Antler Peak site in Mount Rainier National Park. Sapling density ranged from 1120–4413 stems/ha and whitebark pine was present in all sites, but contributed less than 19% of total stems, with densities ranging from 25–343 stems/ha. Subalpine fir was generally the most abundant species, comprising greater than 75% of all stems and having densities of 755–2594 stems/ha. Whitebark pine densities were highest in North Cascades National Park Service Complex in Juanita Lake (343 stems/ha) and Dee Dee Lake (243 stems/ha). Subalpine larch comprised 37% and 28% of Juanita Lake (685 stems/ha) and Rainbow Lakes (727 stems/ha), respectively. In Frying Pan in Mount Rainier National Park, subalpine fir comprised 48%, Alaska yellow cedar 39%, and whitebark pine less than 1% (25 stems/ha).

Seedling densities of all species ranged from 247–14,465 stems/ha, but whitebark pine seedling density only ranged from 3–204 stems/ha, and no seedlings were found in two sites: Palisades (Mount Rainier National Park) and Stiletto (North Cascades National Park Service Complex). Subalpine fir was the most abundant in all sites, but Frying Pan had high densities of Alaska yellow cedar (17%) and silver fir (25%). Subalpine larch comprised 29% of all seedlings in Juanita Lake and Rainbow Lakes in North Cascades National Park Service Complex, where subalpine fir was 65% and 52% of stems density, respectively. Whitebark pine seedling density was highest in Dee Dee Lake (141 stems/ha), Juanita Lake (204 stems/ha), and Rainbow Lakes (145 stems/ha).

3.2. Population Health

3.2.1. Whitebark Pine Trees

We established 64 plots within our 13 study sites (Figure 1) and monitored between 249 and 312 trees (live and dead) in each park with plot densities ranging from one to 86 trees; each year, nine to 13 plots had only one to two trees (Table 2). Blister rust occurred in all but two plots, and mountain pine beetle was only found in five plots in Mount Rainier National Park and seven plots in North Cascades National Park Service Complex at very low incidences (i.e. generally one or two trees per plot or 0.4–6.7% of trees surveyed per year).

Park	Year	Sites (<i>n</i>)	Plots	Whitebark Pine Trees	Whitebark Pine Trees per Plot
Mount Rainier National Park	2004 2009 2015	8 8 8	29 29 29	249 260 258	1–43 1–40 1–39
North Cascades National Park Service Complex	2004 2009 2015/2016	5 5 5	35 35 35	287 295 312	1–79 1–82 1–86

Table 2. Number of whitebark pine trees sampled by year at Mount Rainier National Park and North Cascades National Park Service Complex.

In 2016, only 18% of whitebark pine trees in Mount Rainier National Park appeared free of WPBR infection, 38% were live and infected with WPBR, and 44% of all whitebark pines were dead. In North Cascades National Park Service Complex, 35% of all trees were live and uninfected, 44% were live and infected, and 21% of trees were dead. Tree mortality was highest in Mount Rainier National Park during each survey period while WPBR incidence was higher in North Cascades National Park Service Complex during each survey period. Cumulative mortality increased from 37.8% in 2004 to 43.8% in 2015; average proportion of live infected trees increased from 18.5% to 38.8% (Figure 3, Table S1 Supplementary Material). In North Cascades National Park Service Complex, average mortality in 2004 was 6.7% and rose to 21.4% in 2015/2016 while the proportion of live infected trees increased from 32.3% to 43.6%.



Figure 3. Percent of whitebark pine trees that were dead, WPBR infected live, and uninfected live trees (and standard error within park) in Mount Rainier National Park and North Cascades National Park Service Complex in 2004, 2009, and 2015/2016. Figures (**a**) and (**b**) are cumulative calculations in (**a**) Mount Rainier and (**b**) North Cascades and figures (**c**) Mount Rainier and (**d**) North Cascades are progressive (i.e. previously dead trees were removed from 2009 and 2015/2016).

When we looked at the temporal progression of WPBR infection, the trends in mortality and WPBR infection were similar among parks despite higher baseline mortality (2004) at Mount Rainier National Park. By 2009, mortality was 14.3% at Mount Rainier National Park and 15.9% in North Cascades National Park Service Complex and 7.9% and 8.4%, respectively, in 2015/2016. WPBR infection incidence was also similar, but slightly higher in Mount Rainier National Park in 2016 (Figure 3, Table S1).

Mortality rates in each park decreased from the 2004–2009 time period to the 2009–2015/2016 time period (Figure 4). At Mount Rainier National Park, the annual mortality rate decreased from 2.37% (95% confidence interval (CI) \pm 1.7) to 1.4% (95% CI \pm 1.2). At North Cascades National Park Service Complex, the annual mortality rate decreased from 4.26% (95% CI \pm 1.8) to 1.9% (95% CI \pm 0.8) during 2004 to 2009, and 2009 to 2015/2016. When the period from 2004 to 2015/2016 was evaluated, using no information from sampling in 2009, the annual mortality rate for Mount Rainier National Park was 1.5% (95% CI \pm 0.8) and for North Cascades National Park Service Complex was 2.3% (95% CI \pm 0.9).



Figure 4. Percent newly dead trees across the study period for sites in (**a**) Mount Rainier National Park and (**b**) North Cascades National Park Service Complex. 2004 is graphed at zero to indicate the baseline.

In each park, the majority of whitebark pine trees surveyed were in the smaller diameter classes (Figure 5). In 2004, the number of dead trees was distributed throughout all diameter classes, but with each successive survey, increases in WPBR infection and whitebark pine mortality were primarily restricted to the smaller diameter classes. There were very few live whitebark pine trees greater than 40 cm dbh, but they remained alive and without evidence of WPBR infection in Mount Rainier National Park and infected but alive in North Cascades National Park Service Complex.



Figure 5. Number of whitebark pine trees surveyed by diameter class and health over the three survey periods: (a) Mount Rainier 2004, (b) North Cascades 2004, (c) Mount Rainier 2009, (d) North Cascades 2009, (e) Mount Rainier 2015, (f) North Cascades 2015/2016. Calculations are progressive, numbers of dead trees in 2009 and 2015/2016 are trees that have died since the last survey period.

3.2.2. Saplings

Whitebark pine saplings were documented in every site but in only 58–75% of study plots, depending on the year. Mortality was generally very low: 0.92–8.11% in North Cascades National Park Service Complex and 2.7–3.7% in Mount Rainier National Park. However, WPBR infection incidence was higher in Mount Rainier National Park (24.6–50.8%) than in North Cascades National Park Service Complex (17.1–24.8%) (Figure 6). A total of 431 live whitebark pine saplings were counted in Mount Rainier National Park during the three survey periods: 142 in 2004, 136 in 2009, and 153 in 2015. In North Cascades National Park Service Complex we counted 588 live saplings: 186 in 2004, 192 in 2009, and 210 in 2015/2016. A total of 7721 saplings of eight additional species were counted in Mount Rainier National Park (2421 in 2004, 1839 in 2009, and 3461 in 2015) with density ranges of 520–843 stems/ha/year. In North Cascades National Park Service Complex we counted

5948 saplings of eight species (1733 in 2004, 1611 in 2009, and 2604 in 2015/2016) with densities of 290–321 stems/ha/year.



Figure 6. Mean proportions of dead, live infected WPBR, and uninfected whitebark saplings with standard error, in (**a**) Mount Rainier National Park and (**b**) North Cascades National Park Service Complex.

In both parks, regeneration (saplings and seedlings) was dominated by subalpine fir. Whitebark pine sapling densities generally ranged from 25–343 stems/ha, with the highest densities in two sites in North Cascades National Park Service Complex at Juanita Lake (343 stems/ha) and Dee Dee Lake (243 stems/ha).

3.2.3. Seedlings

We counted 111 whitebark pine seedlings in Mount Rainier National Park over the three survey periods: 48 in 2004, 28 in 2009, and 35 in 2015. In North Cascades National Park Service Complex we found 103 whitebark pine seedlings in 2004, 109 in 2009, and 140 in 2015/2016. Whitebark pine seedlings occurred in all sites in North Cascades National Park Service Complex and all sites except for Frying Pan in Mount Rainier National Park, but seedlings were patchily distributed and only 43% of plots had whitebark pine seedlings. Seedlings of eight other species did occur in all sites for a total of 19,021 seedlings.

Seedlings densities were even lower in North Cascades National Park Service Complex, ranging from 3–204 stems/ha. The highest seedling densities occurred in North Cascades National Park Service Complex at Dee Dee Lake (141 stems/ha), Juanita Lake (204 stems/ha), and Rainbow Lakes (145 stems/ha).

3.3. Landscape Patterns of Mortality, WPBR Infection and Prevalence

3.3.1. Whitebark Pine Tree Mortality

The best model for prediction of whitebark pine percent dead, based on the lowest AICc, included effects of northness, eastness, and park (Table 3). The model contained a random site intercept and was of the form:

Dead (%) =
$$39.8 - 6.3 \times \text{Northness} + 23.5 \text{ Eastness} - 19.6 \times \text{Park} + u_i$$
. (1)

The *p*-values associated with each parameter from the model were 0.72, 0.32, and 0.16 for northness, eastness, and park, respectively. Though none of these *p*-values was individually significant, the model was the most likely given the data. The RMSE value was 27.4 and the bias was -0.7 indicating good predictive performance from the model given the population. The RMSE value of 27.4 was relatively high given the range of the dependent variable from 0–100%, however, we would expect a fair amount of variation among the population having come from several stands in two

different parks. Higher variation or larger RMSE was expected. The model indicated the percent of dead whitebark pine trees decreased with northness and increased with eastness, indicating more dead trees on south-east facing slopes. The percent of dead trees was lower at North Cascades National Park Service Complex than Mount Rainier National Park.

Table 3. Top five linear mixed effect models for predicting percent dead trees; the selected model is in **bold**. All models contain a random site effect. DeltaAICc is the difference in AICc from the top model.

Model Form	AICc	DeltaAICc
Percent Dead ~Northness + Eastness + Park	309.4	0.0
Percent Dead ~Slope + Northness + Eastness + Park	310.1	0.7
Percent Dead ~Elevation + Northness + Eastness + Park	310.7	1.3
Percent Dead ~Northness + Eastness + Park + dbh	311.1	1.7
Percent Dead ~Elevation + Slope + Northness + Eastness + Park	311.6	2.2

We also looked at the third-best model, including northness, eastness, park, and elevation, to evaluate the relationship between elevation and mortality. This model was less than two delta AIC values from the best model and could be considered equally likely given the data [25]. The interpretation of the model coefficients for northness, eastness, and park did not change with the addition of an elevation parameter, though the park coefficient became less significant due to the correlation between park and elevation. The direction of the model coefficient for elevation indicated the percent of dead trees decreased with increased elevation.

3.3.2. WPBR Infection

The best model for percent live infected included effects of northness, eastness, and park (Table 4). The model parameters and form with random site intercept u_i were:

Live Infected (%) =
$$28.8 - 14.0 \times \text{Northness} + 10.1 \times \text{Eastness} + 9.8 \times \text{Park} + u_i$$
. (2)

Table 4. Relative rating for top five linear mixed effect models for predicting percent live infected trees; the selected best model is in **bold**. All models contain a random site effect. DeltaAICc is the difference in AICc from the top model.

Model Form	AICc	DeltaAICc
Percent Infection ~Northness + Eastness + Park	321.9	0.0
Percent Infection ~Slope + Northness + Eastness + Park	322.6	0.7
Percent Infection ~Northness + Eastness + Park + dbh	323.8	1.9
Percent Infection ~Slope + Northness + Eastness + Park + dbh	324.6	2.7
Percent Infection ~Northness + Eastness	326.5	4.6

The *p*-values associated with each parameter from the model were 0.25, 0.52, and 0.30 for northness, eastness, and park, respectively. Though none of these *p*-values was individually significant, the model was the most likely given the data. The RMSE value was 21.5, and the bias was -1.0 indicating good predictive performance of the model given the expected variation among trees in the population. The model indicated the percent of live infected trees decreased with northness and increased with eastness, indicating more infected trees on south-east facing slopes. The percent of live infected trees was also greater at North Cascades National Park Service Complex than Mount Rainier National Park.

3.3.3. Prevalence

The best model for prevalence of blister-rust infected whitebark pines included effects of dbh, dbh^2 and year (Table 5). The model parameters and form with random nested plot and site effect at the intercept u_i were:

Prevalence =
$$-0.5 + 3.1 \times dbh - 2.1 \times dbh^2 + 0.4 \times year + u_i$$
. (3)

Table 5. Relative rating for top five generalized linear mixed effect models for predicting blister rust prevalence; the selected model is in **bold**. All models contain a random nested tree and plot effect. DeltaAICc is the difference in AICc from the top model.

Model Form	AICc	DeltaAICc
prevalence ~dbh + dbh2 + year	1312.7	0.0
prevalence ~dbh + dbh2 + Park + year	1313.3	0.6
prevalence ~dbh + dbh2 + year + dbh:year	1314.0	1.4
prevalence ~dbh + dbh2 + Park + year + dbh:year	1314.7	2.0
prevalence ~dbh + year	1323.2	10.5

The model indicated the relationship between dbh and prevalence was different for each year. Prevalence increased over all diameters of white bark pine trees from 2004 to present (Figure 7). At Mount Rainier and North Cascades National Parks, the model predicts that nearly all white bark pine trees between 15 to 60 dbh are either infected or have already died due to WPBR.



Figure 7. Model predicted prevalence across range of tree diameters (dbh in cm) for each study year.

4. Discussion and Conclusions

The results of our study increase the spatial and temporal resolution of knowledge on the status and trends in whitebark pine population health in the Cascade Mountains. The incidence of infection (18–32%) and mortality (7–38%) that we documented in 2004 were similar in range to those recorded on adjacent forest lands by Shoal and Aubry in 2004–2005 (infection 5–73% and mortality 1–61%) [17] and British Columbia by Campbell and Antos in 1994–1995 (blister rust incidence 27–44% and mortality about 21%) [26]. In all three studies, mountain pine beetle presence was rare and WPBR was thought to be the primary cause of mortality. Over the course of our study, we had increasing confidence in WPBR as the primary cause of mortality because: (1) it was the primary agent of damage to whitebark

pine, (2) evidence of mountain pine beetle was observed in few trees, (3) we saw no evidence of stand-replacing fires, and (4) trees that died after they were tagged in 2004 had bole or stem cankers present in previous surveys.

Although Shoal and Aubry reported north-south and west–east trends in the incidence of blister rust infection reflecting gradients in temperature and precipitation from Washington to Oregon [17], these trends were not apparent in our study. Our study was conducted across a smaller geographic scale and may indicate that environmental conditions are generally favorable for WPBR spread throughout our parks [26]. We found incidence of infection and mortality were higher on south and east aspects and mortality decreased with elevation. Decreasing mortality with elevation could reflect fewer frost free days, hence a shorter growing season for whitebark pine producing a lag between infection and mortality with slower disease progression from needle to tree bole [10,26]. Or, the decrease in frost free days could limit spore production and spread from alternate hosts to whitebark pine [26].

Over the 12 years since we initiated our study, the percent of whitebark pine infected with WPBR and the percent of dead whitebark pine increased in all of our study sites. Although annualized mortality rates decreased between monitoring periods, the prevalence of WPBR infection and mortality increased across our park landscapes. With each survey period, the number of whitebark pine trees in our study sites decreased and natural regeneration was limited. Initially, incidence of WPBR and mortality were highest in larger diameter classes, but with each successive survey, smaller diameter trees also became infected or died. When we modeled prevalence of WPBR infected trees and dead whitebark pine trees on the landscape, we found that diameter and year were the most important variables rather than site or park, and with each survey year the prediction of prevalence increased in all diameter classes.

Our study results indicate that the primary driver of whitebark pine decline in our two parks is WPBR. Ettl and Cottone [27] developed a spatially explicit model of whitebark pine for Mount Rainier National Park using dendrological estimates of growth and mortality and predicted that there would be fewer than 100 trees left in the park in 148 years. As we begin to develop long-term strategies for managing whitebark pine in our parks, we need to consider levels and frequency of genetic resistance to WPBR and the influence of changing climates on stand dynamics [28]. Since WPBR was introduced over 100 years ago, some of the older surviving trees may be indicative of resistance to WPBR. We are screening progeny from cones collected in Mount Rainier and North Cascades at the USDA Forest Service Dorena Genetic Resource Center for resistance and have found moderate levels of genetic resistance in Mount Rainier National Park families and high genotypic diversity in samples from both parks [29]. Progeny of parent trees from Mount Rainier National Park have shown among the highest levels of genetic resistance to WPBR of any geographic sources to date in seedling inoculation trials [30]. But our cone collections have been limited in geographic scope and should be expanded to provide an accurate picture of the frequency of resistance. We also need to conduct field trials to validate the results of seedling inoculation trials to inform restoration prescriptions (Sniezko, per. comm).

In our surveys, we found that whitebark pine was a minor component of fairly diverse tree stands. With each successive survey, the number of reproductive whitebark pine trees decreased and natural regeneration was dominated by subalpine fir. Many sites in our surveys had no whitebark pine seedlings or saplings, and maximum sapling density was only 343 stems/ha. In some sites, density of subalpine fir saplings was over 6000 stems/ha and seedling densities were over 14,000 stems/ha. McCaughey et al. [31] recommended planting hardy seedlings from potentially rust-resistant trees at a density of 479 stems/ha, based on an assumption of 50% survival. The seedling and sapling densities at our sites are not high enough to perpetuate whitebark pine even without considering competition from other species. We do not have enough detailed information from our surveys to ascertain why we have such low levels of regeneration. Visiting sites every five years has not provided enough information on cone crop size or periodicity. Several studies have reported that dispersal by Clark's nutcrackers is a function of the cone density to attract nutcrackers and available canopy openings for seed caching [32–36]. In fact, Ray et al. [37] recently documented that abundance of Clark's nutcrackers

is strongly decreasing in Mount Rainier National Park and appears to be decreasing in North Cascades National Park Service Complex. It would be advantageous if future surveys were conducted more frequently to record cone production and include documentation of canopy openings as a means of evaluating the potential for dispersal of seeds by Clark's nutcrackers.

As we consider management strategies to preserve whitebark pine on our landscapes, the influence of climate change adds to the complexity of our management [28]. Warmer summers and lower snowpack will facilitate the establishment of more trees in subalpine ecosystems [38], increasing competition in the overstory and regeneration stratum. If lodgepole or western white pine distributions expand, mountain pine beetle may become a stronger driver of whitebark pine mortality. Fire frequency, size, and severity are projected to increase with warming climates and may prepare new areas for regeneration, entirely remove stands, or in areas where fire is excluded, it may result in lower densities of whitebark pine regeneration [38,39]. Warmer, drier climates could also influence patterns of mortality and infection if north aspects become more suitable for WPBR reproduction and infection of WBP [35,40].

Patterns in prevalence, mortality, infection, and regeneration are highly variable across our park landscapes, pointing toward the need for increased monitoring, more genetic resistance screening, and development of site specific restoration plans that include active management alternatives such as planting. The National Park Service Organic Act states that the responsibility of the National Park Service (NPS) is to conserve park resources unimpaired for future generations [41]. Loss of whitebark pine, from WPBR, threatens to change ecosystem processes and the trajectory of these ecosystems [1,2]. In 2012, the National Park Service Advisory Board recommended that the National Park Service should " ... steward NPS resources for continuous change that is not yet fully understood ... " to protect ecological integrity while relying on the best available sound science [42].

Whitebark pine was listed as a candidate species by the US Fish and Wildlife Service under the US Endangered Species Act (ESA) in 2011 and as endangered in 2012 under Canada's Species at Risk Act [11,12]. Currently, a collaborative effort to develop a Range-wide Whitebark Pine Restoration Plan is underway [43]. Most of the whitebark pine in the US is distributed on federal lands, and many of these lands are designated Wilderness. NPS policies support science-based management and continuous change in naturally evolving levels of genetic diversity and ecosystem function. Protection of whitebark pine populations may require hands-on restoration tactics that could appear at odds with the paradigm of Wilderness management [44]. Protected lands managers will need to re-examine how Wilderness areas are managed in light of the severity and rate at which introduced species, in combination with changing climates, are altering these landscapes.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/9/5/244/s1, Table S1: Cumulative and progressive average percent of whitebark pine trees that were recorded as dead, live infected, and live uninfected by park and year.

Author Contributions: R.M.R. conceived and designed the study. R.M.R., S.H., L.J., and J.R.B., and L.P.G. compiled the data, and conducted the statistical analysis, and wrote the manuscript. L.P.G., R.M.R., and J.R.B. were responsible for data quality assurance.

Funding: This study has been funded by the National Park Service through the North Coast and Cascades Network Inventory and Monitoring Program and the USDA Forest Service Forest Health Protection Program.

Acknowledgments: We thank the National Park Service field crews for monitoring the plots and entering data and Natalya Antonova for GIS support. We thank Linda Koespell, Steve Gibbons, Catharine Copass, Mark Huff, and three anonymous reviewers for thoughtful review of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

Tomback, D.F.; Arno, S.F.; Keane, R.E. The compelling case for management intervention. In *Whitebark Pine Communities: Ecology and Restoration;* Tomback, D.F., Arno, S.F., Keane, R.E., Eds.; Island Press: Washington, DC, USA, 2001; pp. 3–28.

- 2. Resler, L.M.; Tomback, D.F. Blister rust prevalence in krummholz whitebark pine: implications for treeline dynamics, northern Rocky Mountains, Montana, U.S.A. *Arct. Antarct. Alp. Res.* **2008**, *40*, 161–170. [CrossRef]
- 3. Benedict, W.V. *History of White Pine Blister Rust Control—A Personal Account;* US Department of Agriculture Forest Service: Washington, DC, USA, 1981.
- 4. Hadfield, J. *History of White Pine Blister Rust on Whitebark Pine in the Pacific Northwest;* US Department of Agriculture, Forest Service, Pacific Northwest Region, Forestry Sciences Lab, Wenatchee Service Center: Wenatchee, WA, USA, 2000.
- McDonald, G.I.; Hoff, R.J. Blister rust: An introduced plague. In *Whitebark Pine Communities: Ecology* and Restoration; Tomback, D.F., Arno, S.F., Keane, R.E., Eds.; Island Press: Washington, DC, USA, 2001; pp. 193–220.
- 6. Keane, R.E.; Arno, S.F. Rapid decline of whitebark pine in western Montana: Evidence from 20-year remeasurements. *West. J. Appl. For.* **1993**, *8*, 44–47.
- Kendall, K.C.; Kean, R.E. Whitebark pine decline: Infection, mortality, and population trends. In *Whitebark Pine Communities: Ecology and Restoration*; Tomback, D.F., Arno, S.F., Keane, R.E., Eds.; Island Press: Washington, DC, USA, 2001; pp. 221–242.
- 8. Murray, M.P.; Rasmussen, M.C. Non-native blister rust diseases on whitebark pine at Crater Lake National Park. *Northwest Sci.* 2003, 77, 87–90.
- 9. Schwandt, J.W.; Lockman, I.B.; Klilejunas, J.T.; Muir, J.A. Current health issues and management strategies for white pines in the western United States and Canada. *For. Path.* **2010**, *40*, 226–250. [CrossRef]
- 10. Smith, C.M.; Shepard, B.; Stuart-Smith, J. Changes in blister rust infection and mortality over time. *Can. J. For. Res.* **2013**, *43*, 90–96. [CrossRef]
- 11. U.S. Fish and Wildlife. Endangered and threatened wildlife and plants; 12-month finding on petition to list Pinus albicaulis as endangered or threatened with critical habitat. *Fed. Regist.* **2011**, *76*, 42631–42654.
- 12. Species at Risk Public Registry. Available online: http://registrelep-sararegistry.gc.ca/default.asp?lang=en& n=6B319869-1#2 (accessed on 23 March 2018).
- 13. Gynn, J.C.; Chapman, C.M. *Blister Rust Control, Mount Rainier National Park*; Unpublished Report; Mount Rainier National Park: Washington, DC, USA, 1951.
- 14. Gynn, J.C.; Chapman, C.M. *Blister Rust Control, Mount Rainier National Park*; Unpublished Report; Mount Rainier National Park: Washington, DC, USA, 1952.
- 15. Rochefort, R.M. The influence of white pine blister rust (*Cronartium rubicola*) on whitebark pine (*Pinus albicaulis*) in Mount Rainier National Park and North Cascades National Park Service Complex, Washington. *Nat. Areas J.* **2008**, *28*, 290–298. [CrossRef]
- Goheen, E.M.; Goheen, D.J.; Marshall, K.; Danchok, R.S.; Petrick, J.A.; White, D.E. *The Status of Whitebark Pine along the Pacific Crest National Scenic Trail on the Umpqua National Forest*; General Technical Report PNW-GTR-530; U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station: Portland, OR, USA, 2002.
- 17. Shoal, R.; Aubry, C. *Assessment of Whitebark Pine Health on Eight National Forests in Oregon and Washington;* US Department of Agriculture, Forest Service, Pacific Northwest Region: Olympia, WA, USA, 2006.
- Smith, C.M.; Wilson, B.; Rasheed, S.; Walker, R.C.; Carolin, T.; Shepard, B. Whitebark pine and white pine blister rust in the Rocky Mountains of Canada and northern Montana. *Can. J. For. Res.* 2008, *38*, 982–995. [CrossRef]
- Rochefort, R.M.; Bivin, M.M.; Boetsch, J.R.; Grace, L.; Howlin, S.; Acker, S.A.; Thompson, C.C.; Whiteaker, L. *Alpine and Subalpine Vegetation Monitoring Protocol for the North Coast and Cascades Network*; Natural Resource Report NPS/NCCN/NRR—2012/570; National Park Service: Fort Collins, CO, USA, 2012.
- 20. National Park Service, Branch of Forestry CCC. *Vegetation Cover Survey for Mount Rainier National Park;* Published Report-132562; National Park Service, Branch of Forestry CCC: Washington, DC, USA, 1936.
- 21. Agee, J.K.; Kertis, J. *Vegetation Cover Types of the North Cascades*; Report CPSU/US 86-2; National Park Service, Cooperative Park Studies Unit, College of Forest Resources, University of Washington: Seattle, WA, USA, 1986.
- 22. Hoff, R.J. *How to Recognize Blister Rust Infection on Whitebark Pine*; US Department of Agriculture, Forest Service, Intermountain Research Station: Ogden, UT, USA, 1992.
- 23. Cochran, W.G. Sampling Techniques, 3rd ed.; John Wiley and Sons, Inc.: Hoboken, NJ, USA, 1977.
- 24. R Core Team. *R: A Language and Environment for Statistical Computing;* R Foundation for Statistical Computing: Vienna, Austria, 2015.

- 25. Burnham, K.P.; Anderson, D.R. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, 2nd ed.; Springer: New York, New York, USA, 2002.
- 26. Campbell, E.M.; Antos, J.A. Distribution and severity of white pine blister rust and mountain pine beetle on whitebark pine in British Columbia. *Can. J. For. Res.* **2000**, *30*, 1051–1059. [CrossRef]
- 27. Ettl, G.J.; Cottone, N. Whitebark pine (*Pinus albicaulis*) in Mt. Rainier National Park, Washington, USA; response to blister rust infection. In *Species Conservation and Management: Case Studies Using RAMAS GIS*; Akcakaya, H.R., Burgman, M.A., Kindvall, O., Wood, C.C., Sjogren-Gulve, P., Hatfield, J., McCarthy, M.A., Eds.; Oxford University Press: New York, NY, USA, 2004; pp. 36–47.
- Keane, R.E.; Holsinger, L.M.; Mahalovich, M.F.; Tomback, D.F. *Restoring Whitebark Pine Ecosystems in the Face of Climate Change*; Gen. Tech. Rep. RMRS-GTR-361; Department of Agriculture, Forest Service, Rocky Mountain Research Station: Fort Collins, CO, USA, 2017; p. 123.
- 29. Liu, J.; Sniezko, R.; Murray, M.; Wang, N.; Chen, H.; Zamany, A.; Sturrock, R.N.; Savin, D.; Kegley, A. Genetic diversity and population structure of whitebark pine (*Pinus albicaulis* Engelm.) in western North America. *PLoS ONE* **2016**, *11*, e0167986. [CrossRef] [PubMed]
- 30. Sniezko, R.A.; Kegley, A.; Danchok, R.; Long, S. Blister rust resistance in whitebark pine—Early results following artifical inoculation of seedlings from Oregon, Washington, Idaho, Montana, California, and British Columbia seed sources. In *Proceedings of the IUFRO Joint Conference: Genetics of Five-Needle Pines, Rusts of Forest Trees, and Strobusphere, Fort Collins, CO, USA, 15–24 June 2014*; Schoettle, A.W., Sniezko, R.A., Kliejunas, J.T., Eds.; RMRS-P-76. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: Fort Collins, CO, USA, 2018.
- 31. McCaughey, W.; Scott, G.L.; Izlar, K.L. Whitebark pine planting guidelines. *West. J. Appl. For.* 2009, 24, 163–166.
- 32. McKinney, S.T.; Tomback, D.F. The influence of white pine blister rust on seed dispersal in whitebark pine. *Can. J. For. Res.* **2007**, *37*, 1044–1057. [CrossRef]
- Keane, R.; Parsons, R.S. Restoring whitebark pine forests of the Northern Rocky Mountains, USA. *Ecol. Restor.* 2010, 28, 56–70. [CrossRef]
- 34. Barringer, L.E.; Tomback, D.F.; Wunder, M.B.; McKinney, S.T. Whitebark pine stand condition, tree abundance, and cone production as predictors of visitation by Clark's Nutcracker. *PLoS ONE* **2012**, *7*, e37663. [CrossRef] [PubMed]
- 35. Larson, E.R. Influences of the biophysical environment on blister rust and mountain pine beetle, and their interactions, in whitebark pine forests. *J. Biogeogr.* **2011**, 453–470. [CrossRef]
- 36. Larson, E.R.; Kipfmueller, K.F. Patterns in whitebark pine regeneration and their relationships to biophysical site characteristics in southwest Montanan, central Idaho, and Oregon, USA. *Can. J. For. Res.* **2010**, *40*, 476–487. [CrossRef]
- 37. Ray, C.; Saracco, J.F.; Holmgren, M.L.; Wilkerson, R.L.; Siegel, R.B.; Jenkins, K.L.; Ransom, J.I.; Happe, P.J.; Boetsch, J.R.; Huff, M.H. Recent stability of resident and migratory landbird populations in National Parks of the Pacific Northwest. *Ecosphere* **2017**, *8*, e01902. [CrossRef]
- 38. Littell, J.S.; Raymond, C.L.; Rochefort, R.M.; Klein, S.L. Climate change and vegetation in the North Cascade range. In *Climate Change Vulnerability and Adaptation in the North Cascades Region, Washington*; Raymond, C.L., Peterson, D.L., Rochefort, R.M., Eds.; General Technical Report, PNW_GTR-892; U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station: Portland, OR, USA, September 2014; p. 279.
- 39. Siderius, J.; Murray, M.P. *Fire Knowledge for Managing Cascadian Whitebark Pine Ecosystems*; National Park Service and Crater Lake National Park: Seattle, WA, USA, 2005; pp. 1–44.
- 40. Smith, J.P.; Hoffman, J.T. Status of white pine blister rust in the Intermountain West. *West. N. Am Nat.* **2000**, *60*, 165–179.
- 41. The National Park Service Organic Act. Available online: https://www.nps.gov/grba/learn/management/ organic-act-of-1916.htm (accessed on 30 March 2018).
- 42. United State National Park System Advisory Board. Revisiting Leopold: Resource Stewardship in the National Parks. In *The George Wright Forum*; The George Wright Society: Hancock, MI, USA, 2012; pp. 1–23.
- 43. Whitebark Pine Foundation, National Whitebark Pine Restoration Plan. Available online: http://whitebarkfound.org/national-whitebark-pine-restoration-plan/ (accessed on 30 March 2018).

44. Wilderness Act, 16 USC 1131–1136; PL 88–577. Available online: https://www.wilderness.net/nwps/legisact (accessed on 30 March 2018).



@ 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).