Methods for Surveying and Monitoring Whitebark Pine for Blister Rust Infection and Damage

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I. Rationale for Methods Development

Whitebark pine (*Pinus albicaulis*) is considered a keystone subalpine species, facilitating community development after disturbance, regulating snowmelt, preventing soil erosion, and providing an important food source for many seed-eating birds and mammals, including grizzly bears. However, whitebark pine is declining rapidly in much of its range, primarily due to the introduced exotic disease white pine blister rust (*Cronartium ribicola*). The Whitebark Pine Ecosystem Foundation, along with several U.S. and Canadian government agencies, advocates surveying the extent and intensity of blister rust infection throughout the range of whitebark pine and monitoring the dynamics of the disease as essential steps in effective management. Information obtained from surveying and monitoring will enable managers to plan and prioritize forest areas for restoration activities.

Here, the Whitebark Pine Ecosystem Foundation presents a set of methods for surveying the incidence and severity of blister rust within whitebark pine communities and then monitoring the impacts of the rust over time. Recognizing limits to personnel, time, and budgets in management agencies, we designed these methods to gather critical information efficiently, with the minimum of technical training. If these standardized methods are widely adopted, the collected data will provide critical information that is comparable from place to place and from year to year.

The quality and reliability of field data ultimately depends on training and field experience to recognize blister rust symptoms in whitebark pine with reasonable confidence. In addition, because the symptoms of blister rust vary among the white pines, these field methods probably should not be applied to other white pine species without some modification in symptom description and quantification, and specialized field training. Furthermore, the distribution of some high elevation species, such as the bristlecone pines, may be extremely patchy, requiring some modification of these methods or other sampling strategies.

The methods presented here were initially developed after a review of 10 relatively recent surveys of blister rust incidence in different parts of the range of whitebark pine (Appendix I). These surveys used different methods for sampling whitebark pine communities, and collected and analyzed data differently as well. They were conducted by individuals with different backgrounds, including pathology, wildlife ecology, and forest ecology. The objectives of the studies varied, such as determining the presence and incidence of blister rust, general forest health surveys, and the local occurrence of whitebark pine. The methods that appeared to be the most efficient and effective for the purposes of both surveying and monitoring rust infection dynamics were those developed by Keane and Hoff (1991) and modified by Kendall (1994), and further modified and applied in large-scale surveys by Smith and Hoffman (2000a) and Zeglen (2002). After discussion with several resource managers, forest ecologists, and pathologists, we identified the most important variables to measure, requiring the least technical background. Recognizing that the data gathered would be insufficient for technical research regarding the dynamics of blister rust and its hosts, we suggest several add-ons to our methods, depending on the specific research questions asked.

In June, 2003, we conducted a two-day field trial of the draft methods in an area with high blister rust infection incidence in eastern Idaho on the Caribou-Targhee National Forest. Joining us on the field trial was a team of researchers and managers with diverse backgrounds. Our goals were to determine the practicality and efficiency of the methods and to make further revisions in procedure and data collection. We were particularly concerned about the time required to set up a plot and complete the survey, and the justification for all data collected. Additional modifications to the methods have been made following the June, 2004 workshop, "Monitoring Whitebark Pine for Blister Rust: A Methods Workshop," organized by the Whitebark

Pine Ecosystem Foundation. These recent revisions are based on feedback and suggestions from field teams consisting of workshop attendees and instructors, as well the need for compatibility with FS-VEG and current statistical sampling approaches. In the latter case, fixed areas are considered basic sampling units as opposed to minimum numbers of trees.

The Whitebark Pine Ecosystem Foundation will continue to revise these methods over time with the intent of clarifying procedures, variables, and datasheets. We are striving for clear, concise, and logical guidelines which are accessible to resource managers and technical personnel. Although specific objectives may vary with each agency and its mandates and constraints, region of interest, and bounded unit to be surveyed, above all we strongly advocate that data be collected generally following these methods and guidelines, so they may lend themselves to range-wide compilations, and regional comparisons of assessments.

Background

White pine blister rust, an invasive fungal disease native to Eurasia, was inadvertently brought to North America about 100 years ago and has ago and has rapidly spread across most of the range of western five needle white pines. Blister rust infects only five-needled white pines (family Pinaceae, genus *Pinus,* subgenus *Strobus*), and requires gooseberry and currant shrubs (genus *Ribes*) as its alternative host in a complex life cycle involving five spore forms (Hummer 2000, McDonald and Hoff 2001). Basidiospores, produced on *Ribes* leaves, enter the stomata of pine needles, and the fungus then grows down stems into branches and boles. After two to three years the rust produces a sporulating canker, which may girdle and kill the branch or entire tree. Branch cankers may progress to bole (trunk) cankers, which are usually fatal. The low levels of natural resistance in North American five-needled white pines, coupled with favorable climatic conditions, enabled the disease to spread rapidly. Recent observations (P. Zambino, USDA Forest Service, Rocky Mountain Research Station, personal communication) suggest that plants of genus *Pedicularis* and *Castilleja* may also serve as alternate hosts.

Independent introductions of blister rust to both the east and west coasts ultimately resulted in a continent-wide epidemic of this destructive disease. To date, blister rust infects eastern white pine (*Pinus strobus*), western white pine (*P. monticola*), sugar pine *(P. lambertiana*), whitebark pine, southwestern white pine (*P. strobiformis*), foxtail pine (*P. balfouriana*), limber pine (*P. flexilis*), and Rocky Mountain bristlecone pine (*P. aristata*), with the potential to infect Great Basin bristlecone pine (*P. longaeva*) (McDonald and Hoff 2001, Blodgett and Sullivan 2004). Losses of these white pine ecosystems collectively represent significant reductions in forest biodiversity, especially considering geographic variation in habitat types, and the array of successional stages, understory plants, invertebrate and vertebrate species, and microbial and fungal communities that they harbor (Tomback and Kendall 2001, Tomback 2003, Tomback and Achuff in prep.).

Whitebark pine is a species of particular concern with respect to its susceptibility to white pine blister rust (Hoff et al. 1980) and rate of decline in the northwestern United States and southwestern Canada (Kendall and Keane 2001, Tomback *et al.* 2001). While most research and resources in the western United States were focused on the commercially valuable western white pine and sugar pine, at the same time blister rust was spreading into upper subalpine elevations and across the range of whitebark pine. Blister rust is widespread throughout Canadian whitebark pine populations (Stuart-Smith 1998, Campbell and Antos 2000, Zeglen 2002, Smith et al. in prep.), and occurs nearly everywhere within the range of whitebark pine in the United States with the exception of parts of the Great Basin (Kendall and Keane 2001, Smith and Hoffman 2000b). It was previously thought that the more arid regions of the West were unsuitable for spore-formation and spread in blister rust, but the recent detection of blister rust in whitebark pine in the Carson and Jarbidge Ranges of the Great Basin and the progressive

spread of blister rust in limber pine to eastern Wyoming, the Black Hills, and northern Colorado has dispelled that notion (Smith and Hoffman 2000b, McDonald and Hoff 2001 and references therein, Tomback 2003, J. T. Hoffman, personal communication).

The on-going losses of whitebark pine have disturbing implications for both western biodiversity and ecosystem services in particular. First of all, among western five-needled white pines, whitebark pine is geographically most widespread, ranging from 55^0 to 37^0 N and from 128^0 to 107^0 W (McCaughey and Schmidt 2001, Tomback and Achuff, in prep.). This area encompasses tremendous biodiversity, with local and regional geographic variation in whitebark pine community types, disturbance regimes, successional stages, understory species, and wildlife. In addition, whitebark pine is an important food source for granivorous species, including a diverse number of small birds and mammals (Tomback and Kendall 2001). In the Greater Yellowstone Area, and also along the Rocky Mountain Front, whitebark pine seeds are an important pre-hibernation food for grizzly (*Ursus arctos*) and black (*Ursus americanus*) bears (Kendall 1983, Mattson et al. 2001). Because whitebark pine is highly stress-tolerant, it often grows alone at the highest treeline elevation, regulating snow melt, and reducing soil erosion with its root systems. Whitebark pine seeds are dispersed by Clark's nutcrackers (*Nucifraga columbiana*), often over long distances into newly burned areas and other disturbed sites. The robust, hardy seedlings are tolerant of exposed sites and poor seedbeds, including ashy soils with low water-holding capacity. Consequently, whitebark pine is a pioneering species after disturbance, ameliorating the environment for other forest and understory species and facilitating community development (Tomback *et al.* 2001).

In the absence of hands-on management, whitebark pine will continue to decline across the western landscape, replaced in some areas by shade-tolerant species such as Engelmann spruce (*Picea engelmannii*), and subalpine fir (*Abies lasiocarpa*) (Keane *et al.* 1990, Keane 2001). At the highest elevations, it may not be replaced, because other competing conifers are intolerant of stressful conditions, and unless those severe environmental conditions are moderated due to global climate change.. If whitebark pine losses are not reversed, the western forest landscape will become more homogeneous, and in many northern fire-prone areas, stand-replacing fire regimes will replace mixed severity regimes (Keane 2001). Losses in both biodiversity and ecosystem services pertaining to run-off and water quality, are anticipated, and the long-term security of grizzly bear populations in the Greater Yellowstone Area will be threatened.

II. Preliminary Information and Sampling Strategy

Surveying vs. monitoring for blister rust

We regard **surveying** as a one-time inventory effort to collect data on the presence and severity of blister rust within a sampling unit or "plot," and the resulting condition of trees. These data enable managers to determine whether blister rust is a management issue, and, if so, to prioritize areas for restoration action or monitoring. This cooperative effort will contribute to the range-wide database being developed by USDA Forest Service, Forest Health Protection, and other U.S. and Canadian government agencies.

Monitoring involves repeated visits to the same sampling unit over time (e.g., every 5 years) to measure changes in the occurrence and impact of blister rust, using identical methods each time. Monitoring provides much-needed information on the rate of spread and intensification of blister rust within sampling units. We urge that all plots be permanently monumented (see **Setting up the transect**), so they can be relocated and resampled (monitored) in the future. Situations have already arisen where funding becomes available for

resampling previously measured sites, but the sampling units were insufficiently monumented initially and the remeasurements are thus inaccurate.

When to survey and monitor

The time of year selected for blister rust surveys may affect the reliability of the data collected, given that the most important objective of this sampling method is the correct identification of blister rust on trees. The definitive symptom of white pine blister rust is the sporulating canker. A sporulating or active canker is characterized by several to hundreds of orange spore sacs known as "aecia," often referred to as aecial blisters (which explains the common name "blister rust"). These are produced by active cankers in spring, and are generally visible from about May through July and as late as mid-August. Aecia fade in color with time, turning cream-colored or white by the end of the summer. Once the spores release, only small flaps of white tissue remain of the spore sacs. Early in summer, aecia produced by cankers in upper branches may be visible either to the naked eye or with the aid of binoculars, depending on canopy density and height; these active cankers may be easier to overlook by mid to late summer. Because whitebark pine occurs at upper elevations, field crews have a narrow window for reliable surveys—namely between the time snow melts sufficiently for access to high country and before aecia fade. We recommend that blister rust assessments be completed by mid-August for the most consistent identification of blister rust.

Personnel and training requirements

We recommend that field crews (sampling teams) consist of two to three people, with at least one person trained to recognize blister rust symptoms in whitebark pine and experienced in sampling methods presented here. In addition, it is essential that team members be able to identify forest tree species, and particularly whitebark pine, as well as dominant undergrowth species. In geographical regions where limber pine and whitebark pine may co-occur, at least one team member must be familiar with the morphological differences in male and female cones between these two white pines. Furthermore, we recommend that sampling team personnel are completely familiar with the use of all field equipment listed below.

Field equipment

- GPS (Geographic Positioning System) unit (UTM function preferred)
- US Geological Survey Topographic Quads or equivalent maps
- 2-3 pairs binoculars (at least 8 power; 10 x 35 or 10 x 50 recommended)***
- 100 ft or 200 ft (50 m) transect tape
- 15 ft (5 m) metal tape or 100 ft or 50 ft (50 m or 30 m) transect tape in addition to above (for transect width)
- transect stakes, chaining pins, or surveyors flags to secure transect tape
- DBH (diameter at breast height) tape in appropriate measuring system, English (Imperial) or metric
- several rolls of colored surveyor's ribbon
- small plastic calipers (quick to use for small diameter trees)
- clinometer or other measuring device for slope steepness
- compass, adjusted for true north
- 100 surveyor's wire stem flags and rolls of surveyor's tape

- clipboard with plot sheet storage
- plot data sheets on waterproof paper
- fine point permanent markers and graphite pencils
- masking tape
- numbered aluminum tree tags, tag wire (for rebar), and aluminum nails
- ½ inch (1.3 cm) diameter rebar stakes, 1.5 to 3 ft in length (0.5 - 1 m)
- hammer for driving in rebar stakes and aluminum tree tags
- camera

***Good binoculars are essential for examining trees for blister rust symptoms**.   Low quality binoculars (i.e., having poor optics with low light-gathering power) will result in an underestimate of blister rust infection incidence for large trees.

Data collection: field sheets and database

A sample datasheet format is provided for field work in Appendix II. The data sheets may be used as a master for copying onto waterproof paper (available through most forestry catalogs) that can be stored in a covered clipboard during fieldwork. We recommend using fine point permanent markers for good weather, but these will not work under conditions of high humidity and precipitation. Pencils are the best back-up and write well on waterproof paper.

Database software, *Whitebark Pine Blister Rust Survey: Database Application,* Beta Test Version 0.20, was designed specifically for these methods for data storage and summary calculations. This Microsoft Access application was developed by David Pillmore and Brent Frakes, Rocky Mountain Network Inventory and Monitoring, USDI National Park Service, Ft. Collins, Colorado. Information for obtaining the most current software version may be found on the Whitebark Pine Ecosystem Foundation website (www.whitebarkfound.org).

Selecting stands for surveying and/or monitoring: sampling strategies

Background. **Definition:** "A **stand** is a spatially continuous group of trees and associated vegetation having similar structures and growing under similar soil and climatic conditions" (Oliver and Larson 1990). Many, but not all whitebark pine communities meet this definition. In the upper subalpine zone, communities may occur in small patches on the landscape, forming a mosaic of habitat types.

Guiding principles. By the beginning of the field season, we assume that management agencies will have decided which regions should be sampled to determine blister rust infection severity. The geographic scale of area selected for sampling will clearly depend on the resources available, and may vary from an entire region or bounded unit, such as a National Park or National Forest, to a smaller landscape unit, such as a watershed. Once a geographic region has been selected for sampling, the distribution of sample stands within an area should be considered carefully. Ideally, each major landscape feature (ridge or mountain) within the region or unit should be sampled:
- If resources permit, there should be a plot in each major stand (defined as >10 ha) on all landscapes within the sampling region. Plots should be established in the largest stands on the ridge or mountaintop, or more specifically, the stands that are most representative of that entire landscape unit.

- If resources exist for additional sampling, then additional plots should be established on the ridge or mountaintop, with representative forest types as the guide. For example, if resources exist for establishing three plots per landscape unit, then the three most representative stands on the landscape should be sampled, using areal extent to prioritize stands. *Representativeness* can be evaluated based on major vegetation classification categories such as cover type, habitat type, or structural stage.

Previous experience suggests that the percentage of trees infected by blister rust (incidence) within an area may vary with slope aspect (azimuth), elevation, successional stage, tree density, and habitat type, which may co-vary with the aforementioned factors. If support resources are sufficient, we recommend that stands for sampling within landscape units be selected to capture as much diversity in these variables as possible (see Table 1).

Sampled stands should be delineated to encompass conditions as homogeneous as possible with respect to the variables presented in Table 1. Aspect as a variable implies that all trees within a single sample should be generally on the same aspect, such as north or southwest (see **Belt transect plot** for clarification). Elevation as a variable requires that sampling be across a slope contour and not vary much up or down slope. Tree density should also be reasonably consistent, although it ends up averaged across an entire sample. Habitat type or cover type should not change within a sample. All of these are suggestions that will allow for ecological comparisons.

Table 1. Variables for selecting stands for sampling

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Aspect	north	south	east	west
Elevation	low	mid	high	
Successional stage	early	mid	late	
Tree density	low	medium	high	
Habitat type				

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If only a few stands are sampled within a region, the most representative types with respect to the above variables should be selected. However, the most complete sampling regime should begin by identifying the major variation in whitebark pine communities within a given area based on prior vegetation surveys, including GIS (Geographic Information System) layers, and then distributing stands for sampling across the range of variation. This should determine how many stands are sampled. Whenever possible, each stand type should be replicated.

We suggest that stands of mature (cone-bearing) trees be prioritized for sampling, and that stands in early successional (seral) stages with seedlings and saplings be avoided, unless sampling these young communities meets specific objectives.

In the interest of time, workers might be tempted to sample stands that are accessible by road or trail, but the level of infection in a small sample of stands, particularly those stands bordered on one side by a road or trail, may not be representative of the general area. <u>Plots should be located at least a short distance (minimum of 100 ft or 30 m) from a road or trail, and even farther (e.g., 150 ft or 50 m) from a two-lane road.</u>

Selecting a sampling strategy

There are two steps to sampling whitebark pine stands for rust infection and severity. First, the **sampling strategy** guides where and how many plots to establish on the landscape, and, ideally, when to remeasure plots if monitoring is possible. Then, the **sampling methods**

determine what is actually measured or estimated on each plot. Thus, the sampling strategy explicitly describes where, when, and how the sampling methods are implemented across the landscape. This section describes options for designing a sampling strategy to measure rust infection and damage across a landscape.

__Definitions__. First, we define the **sample plot** as the **sampling unit**, and the primary objective of the sampling effort is to determine the percentage blister rust infection, and resulting tree mortality and damage, at the plot level. The sampling environment may be viewed hierarchically. At the coarsest scale, we have the **sampling region**, which is defined as a very large geographical area with similar whitebark pine ecosystem dynamics, because of similar climate and habitat types. The **sample landscape** is that portion of the sampling region defined by the presence of whitebark pine. For example, the northern Rocky Mountains of the United States might be considered a sampling region, and the extent of whitebark pine in the Bitterroot Mountains might be considered one sample landscape within the region. The size of the region or landscape under consideration depends on the objective of the sampling effort. Within the sample landscape, we must identify **sample stands**. Sample stands are selected based on some form of biophysical stratification of the sample landscape. For example, sample landscapes can be spatially stratified by habitat types, cover types, elevation zones, aspect classes or any other biophysically based classification or classification combination (see Table 1). The more stratification variables, the higher the number of sample stands. The degree or complexity of stratification undertaken in sampling depends mostly on the resources (personnel, time, and funding) available to conduct the sampling; so fewer stratifications are used when funding is low. Sample plots) are established within the **sample stands**.

__Approaches to sampling design: statistical vs. relevé__. There are two approaches commonly used to design a sampling strategy. The first is a **statistical approach** where plots are randomly established within all or some sampling stands across the sample landscape. Sample stands are selected according to the stratification protocol mentioned previously. The number of plots placed in a sample stand depends on the variation of blister rust infection and damage across the plots within a stand. This, of course, requires an estimate of the infection variation before the beginning of the sampling effort (statisticians call this *a priori* knowledge) to compute the number of plots to establish within a sample stand. If rust infection rates are highly variable (i.e., percentage infected trees within plots is highly variable across plots), then a large number of plots are needed within the sample stand to accurately describe that variation. Many statistical textbooks are available for reference to compute the number of plots from a variety of methods (we recommend Elzinga *et al.* 2001).

The statistical approach is used when data must be compared using rigorous statistical assumptions and procedures across space *and* time. It requires more funding, personnel, and time resources. Many people use the statistical approach when rigorous comparisons over time are desired or when comparison of sampling units must include an estimate of error and variance across the sample stand or landscape. For monitoring, the sampling units all must be revisited and in the same timeframe for useful statistical comparisons. We strongly recommend consulting with a statistician before proceeding with a statistical approach. Some land management agencies do not have the resources to implement this sampling approach. So, the question remains, how do we sample a landscape when limited funds do not allow for a statistical approach?

The **relevé approach** provides a less demanding alternative to the rigorous statistical approach. The relevé approach is used when documentation of important ecological changes is more important than statistically valid estimates of change. In the relevé approach, one plot is placed in a representative portion of the sample stand "without preconceived bias" (Mueller-

Dombois and Ellenberg 1974). *Representativeness* is evaluated based on stand history, vegetation composition, stand structure and a host of other ecological attributes (see Table 1). A statistical summary of relevé plots is possible when more than one plot is established within a stand, but this summary has limited statistical rigor because of subjective plot establishment.

The advantage of this method is that the manager can choose where to place the plots based on past experience, management objectives, and crew safety. The disadvantage of this approach is its obvious location bias and lack of replication within a sample stand; also, plot locations can conceivably be manipulated to influence monitoring or inventory results. In general, the relevé approach is used when time, money, or personnel limitations require the sampling to be done quickly but without comprising the temporal aspects of monitoring. Relevé sampling is used when description is more important than a statistically valid spatial comparison, and when plots must be established in certain communities or stands because of management objectives.

There are clear limitations to the relevé approach: First, variation within stands is not quantified, so a statistical comparison across stands (comparing one stand to another) cannot be done. Next, the variation of important elements, such as the proportion of trees infected by blister rust, or differences in regeneration density, are not measured across space, so a statistically valid landscape comparison is not possible. The only possible statistically valid comparison in the relevé method is the comparison of one single plot measure at two time periods, but that has limitations as well.

It is possible to increase the robustness of sampling within a stand, however. If the comparison of a plot over two time periods indicates an increase in blister rust infection and tree damage, more plots can be established in the stand for future monitoring and more rigorous statistical comparisons.

Designing the sampling strategy.
The first step in designing a sampling strategy is to identify the specific objectives of the sampling effort. Here, the objectives might be "determining the level of infection and damage in whitebark pine stands". However, we must also decide if the field measurements are to describe current conditions (i.e., inventory) or to document changes in rust-pine dynamics over time (i.e., monitoring). The next step is to identify the amount of resources (money, time, personnel, vehicles, etc.) available to accomplish the sampling task. Once resources are known, then the sampling approach can be selected. If the objective asserts that statistical validity and significance are critical *and* there are enough resources to accomplish this task, then a statistical approach is warranted. However, if resources are limited, then a relevé approach might be indicated. The following discussion will help with this decision.

The exact amount of resources available to conduct the rust sampling may be evaluated to aid the selection of a sampling strategy. There are four types of resources that should be considered: 1) funding, 2) personnel, 3) logistics, and 4) time. All of these resources are related, but each resource should be carefully appraised to determine its contribution to the monitoring project. We must be sure to account for the extent and complexity of the sampling region, including time required to reach more remote areas, in the assessment of sampling resources.

Computation of **sampling potential (SP or number of plots)** estimates the number of plots that can be installed during the sampling effort. This statistic integrates most sampling resources into one index that describes the capacity to perform the sampling project. The sampling potential (SP) is the project funds (PF or dollars) divided by crew costs (CC or dollars per day) multiplied by plot production rate (PPR or number of plots per day).

$$SP = \frac{(PF)(PPR)}{CC}$$

Since it is difficult to obtain a good estimate of plot production rate for this effort, a default value is estimated here for use until more accurate measurements are available. We assume a crew of two people can complete the sampling methods for a single plot in about 1.5 hours, and it takes about 2 hours round trip to get to the sample stand. Under these conditions, the PPR is about 4 plots; under more challenging conditions, PPR could be 1 plot. The project funds (PF) are usually fixed for the project, and the crew costs (CC) would be the cost of 2 technicians and their vehicle for one day, which we estimate at about $350 per day. So, if $10,000 are available for sampling rust infection and we use the default PPR and CC estimates, then the sampling potential (SP) may be as high as 114 plots or $10,000*4/$350 or as low as 28 plots. We must remember that this is a coarse estimate of sampling potential and can be improved with experience with this sampling method.

The **sampling region** and the **sample landscapes** must be identified in the next phase of sampling design. Typically, the region is a large geographic area where whitebark pine ecosystem dynamics are somewhat constant, such as the Greater Yellowstone Ecosystem, northern Rockies, or north Cascades. However, these regions can also be delineated by political boundaries such as Flathead National Forest, Glacier National Park, or Mission Mountain Wilderness area. The sample landscapes are the divisions of land within the region that can be used for sampling stratification. We suggest that the region be divided by lands with the potential to support whitebark pine. This is often done by creating a GIS layer that shows all lands above a minimum elevation that would correspond to the lower elevation limit of whitebark pine for that region. However, the region can also be divided into watersheds or square grids if desired. Next, the number of sample landscapes within the sample region is determined. If this number is large (> 50), then only a portion of these sample landscapes can be sampled, depending on resources available. A good rule of thumb is to sample at least 20 percent of the total landscapes. The number of sample landscapes estimated (designated "NSLS" for number sample landscapes) is compared to the sampling potential (SP). If NSLS is equal to or greater than SP (i.e., there is not enough money to sample every identified sample landscape), then a relevé approach might be the best alternative. The other possibility is to decrease the number of sample landscapes. On the other hand, if NSLS is much less than SP, then a statistical approach might be possible. But, there is still some information we need to finish our sample design selection.

Often, the variability of rust infection and damage across a whitebark pine landscape is so great that it is important to stratify the landscape by those biophysical characteristics that are most influencing rust dynamics. Past studies have stratified sample landscapes by habitat type, elevation zone, aspect, cover type, stand structure or combinations of these depending on the sample region. Stratification is very important in identifying the causal mechanisms responsible for rust infection. If this is an objective, then one or more forms of landscape stratification must be selected and imposed on the sample region. For example, this might involve mapping habitat type from elevation zones and aspect classes using GIS techniques. Once the sample landscapes are mapped with the selected stratification criteria, then an average number of stratification combinations must be computed across each sample landscape in the sample region. The stratification combinations (e.g., south aspects above 8200 ft or 2500 m elevation) delineate land areas that correspond to **sample stands** in this approach. This average number of sample stands per sample landscape is then multiplied by the NSLS to get total number of sample stands (NSS). The NSS is then used to describe the sample population.

If NSS is larger than SP, we suggest the relevé approach be used, and describe the sample design in the next sections. However, if resources are adequate for a large number of plots (SP >> NSS, e.g., SP is three times greater than NSS), then the sampling effort can be designed around a statistical approach. Because statistical design is often complex, it is not possible to discuss all aspects in this paper. We recommend that managers work with a statistician to design an approach tailored to their specific goals and land area.

Implementing the relevé approach.

With the relevé approach, plots are located in a representative portion of the sample stand without preconceived bias. First, the stand is explored to estimate the entire range of vegetation and biophysical conditions. Based on this reconnaissance, a site within the stand is selected that reflects modal conditions across the entire stand. Representative conditions should be assessed from a wide range of ecological attributes: species composition, vertical stand structure (e.g., canopy layers), tree size (e.g., DBH, 4.5 ft or 1.4 m, and height), and tree health. Next, an assessment of the biophysical environment should be used to judge *representativeness.* Here, the sample site should represent modal topographic conditions or average slope, aspect, slope position, and elevation attributes. Any disturbance evident in the macroplot (i.e., insect, disease, fire, browsing) must represent the entire site.

Ideally, each stand on the landscape should be sampled under the relevé approach, but this is rarely possible. Some stands are inaccessible or remote, or there are not enough resources to sample the entire landscape. Therefore, a compromise must be struck between sampling distribution and logistics, where the number of samples is maximized, given the resources available. Usually, only a fraction of the landscape can actually be sampled, so the most challenging part of relevé sampling is to decide which stands to sample.

Perhaps the most important factor in sample stand selection for relevé sampling is to ensure that there are enough plots across all categories in the spatial stratification (i.e., mapped categories such as cover types). We should be certain that each mapped category has sufficient plot representation. But, strict adherence to any sampling rule, such as a balanced plot representation, is often impossible in nature due to many unforeseen obstacles and situations, such as a sudden snowstorm or 30 successive days of rain.

Sometimes, cluster sampling is more efficient than random sampling, especially for large landscapes. Cluster sampling occurs when adjacent stands are identified for sampling around an easily accessible point rather than distributed randomly throughout the landscape. Cluster sampling minimizes transport time, while sampling the required map categories by area or frequency. The main disadvantage of cluster sampling is the introduction of bias by sampling in a small area and extrapolating those measured conditions over the large area, and bias from the subjective location of the cluster center. Cluster sampling is a great way to squeeze the maximum amount of information from the sampling effort, saving costs. However, one cluster should never be used to describe conditions across a large landscape. Instead, clusters should be sprinkled geographically around the sample landscape using transportation routes (e.g., roads, trails, rivers) as cluster centers. Cluster sampling does have an element of subjectivity in the placement of cluster centers, but when sampling resources are low, cluster sampling is a valuable alternative to random sampling.

To summarize the relevé approach, we suggest that one plot be established per sample stand on a subsample (20 percent) of the sample landscapes within the region. The sample landscapes may be selected at random, or based on accessibility, safety, and crew experience.

Implementing the statistical approach.

The statistical approach is appropriate when it is important to compare differences across entire stands or to detect changes over time within a stand or across stands with statistical rigor. The statistical approach attempts to quantify the

variance in a wide variety of sampled plots *within* a sampled stand. **In other words this approach allows more powerful statistical comparisons.** Consequently, it has strong interpretative power, but it comes at a cost. It is often resource-intensive to implement a statistical approach, because multiple plots per stand are required.

Designing a viable statistical sampling scheme requires extensive expertise in statistical sampling techniques, field sampling, and operational management. As a result, this section is only a starting point for statistically based sampling and is not intended as a complete reference on the subject. In fact, we strongly recommend that you design your sampling scheme with a statistician or sampling expert.

Since multiple plots are needed to quantify the variance in rust-related characteristics, the first important step in the statistical approach is to determine the number of plots needed to adequately sample each sample stand. Most statistical sampling techniques require that this be determined by the amount of variability in the rust infection and damage characteristics being sampled using standard formulae. This is somewhat perplexing, because we often do not have a good estimate of variability to begin with. There are formulae presented in statistical texts to help determine the number of plots required (e.g., (Elzinga *et al.* 2001). As a rule of thumb, there should be at least three plots per sample stand and at least one plot per 25 acres (10 hectares) of sample stand (i.e., 100 ha sample stand has 10 plots).

Sample stands should be selected with respect to a sampling stratification strategy. Within sample stands, plots should be randomly established using high resolution maps or photos with ruler, compass, and a pencil. Within a sample stand, plots can be selected at random with a grid overlay as follows: Two random numbers are generated by calculator or computer. The first random number is multiplied by the number of rows, and the second by the number of columns. The two products generate coordinates for a cell in the grid where the plot will be located. This point is then located on the map, and the procedure repeated for all plot locations. With all plots identified within a sample stand, a line can be drawn from an easily identified starting point (road intersection, river confluence, trail switchback) to the nearest plot. The compass bearing and distance can be determined from the line, with the ruler and a compass. Next, a line can be drawn from the first plot to the next nearest plot, and distance and azimuth again determined. This procedure is repeated for all plots in the sample stand so they are each connected by a traverse. These points should be input into a GPS unit as labeled waypoints; and, in the field, surveyors can use navigation features to direct them to the starting points.

__Monitoring vs. inventory__. The above discussion of sampling design can be applied to both inventory and monitoring with some caveats. It is important to realize that the relevé approach can only be used to describe current conditions (inventory) or changes in those conditions (monitoring). It cannot be used to determine if these changes are statistically significant. To determine with rigor whether rust infection is different across sample stands (inventory) or is increasing or decreasing within a sample stand (monitoring), a statistical approach is absolutely needed. The power of the statistical method is directly related to the number of plots established within the sample stands on the sample landscape. Thus, it is critical to reconcile the sample design with the stated objectives before actually implementing the sampling project.

III. Establishing and Sampling Plots

The belt transect plot as a sampling unit

As described previously, the sampling unit is the sample plot. We recommend that a belt transect of fixed width and length be used for constructing a sample plot. We consider the belt transect plot the easiest sampling unit to construct and relocate. First of all, the transect line serves as a baseline that can be monumented at two ends, providing some redundancy for relocating the plot. Second, the survey team can quickly establish the belt widths while simultaneously marking trees that are included within the plot but at belt limits, increasing efficiency. Third, the belt baseline and shape of the plot enables the plot to follow a contour line with minimal variation in elevation and aspect. Fourth, if the selected sampling regime requires a minimum number of trees, then it is easy to add length to the transect to accommodate more trees, although we strongly recommend the fixed area plot. We have heard arguments for different shapes, such as circular plots in extremely patchy environments. Although we suggest that the methods presented here can be followed in most circumstances, different plot shapes are compatible with these methods, as well as the companion database software, particularly if plot areas remain the same. The start points for belt transect plots should be roughly predetermined, as described above for either statistical or relevé sampling.

Setting up the belt transect plot

Note: All belt transect plots should be treated as if intended for monitoring over time. The start and end points should be permanently monumented (see below), and all whitebark pine trees above 4.5 ft (1.4 m) tall within the plot should be labeled with a numbered aluminum tree tag. For monitoring purposes, it is essential that when the belt transect plot is reconstructed, the plot includes *the same trees that were originally described.* Otherwise, the data collected in subsequent surveys are of questionable value. A written description of how to find the starting point of the transect plot with respect to the hiking trail or road and nearby landmarks is essential for relocating the plot. Marking sampled stand locations on a USGS topographic map in addition to taking GPS (Geographical Positioning System) locations will facilitate mapping and relocating sampled stands. We recommend that several photos be taken of the transect origin and of the belt transect to show the general condition of the stand.
Note: Before the **start point** is determined, the sampling location should be examined in its entirety to make certain that the forest is reasonably homogeneous with respect to structure and habitat type and will accommodate a plot without a major change in aspect, slope steepness, or elevation. We emphasize that this "walk through" is very important for preventing mistakes and lost time. Consistency is also recommended for setting up transect plots. Facing uphill, we suggest that all transects be directed from right to left—start point to end point. At the starting point of the transect line, the left side would always be the downhill side.

Monumenting the belt transect plot. The start and end of the completed transect line should be comprehensively monumented to ensure that future crews can reconstruct it. We suggest that this be done *after* the transect baseline has been established (see below) for a number of reasons. *We strongly recommend that four types of monumenting techniques be used for each plot*.
At the start point (the beginning of the transect): First, the origin (zero point) of the transect should be monumented with a 1.5 to 3.0 ft (0.5 to1.0 m) long iron rebar stake driven into the ground half to two-thirds its length.   A steel or aluminum tag is wired to this rebar stake near the top, and the tag number recorded on the data sheet. Second, photos are taken to

document the appearance of the plot: One photo is taken of the starting rebar stake, sighting down the transect line; and, a second photo is taken perpendicular to the transect line, starting at the left side and looking right through the starting rebar stake (with picture numbers listed on data sheet). If the left side is consistently the downhill side, the second photo reflects what the crew sees walking uphill. For consistency, we suggest that the left side of the start point always be on the downhill side. This procedure should be repeated at the end point of the transect line, once it is established (see below) with one photo sighted back along the transect line from the end monument, and a second photo taken from the right side through the end rebar towards the left side.

At the end point (the end of the transect line): If the transect plot is a fixed area plot, the end point will be identified during initial set-up. However, if the survey method selected is based on a minimum number of trees, only after the plot has been established and completely sampled will the final end point of the transect line be known. In either case, the end point of the transect line should be marked with a rebar stake, with a numbered tag wired to the top; the number is recorded as a backup (in case either the start monument tag is lost or tree tags are lost). The location of the end stake is geo-referenced with a good GPS instrument and recorded. The two end photos should be taken at this point.

Photos should be taken after the transect line has been laid and the belt width flagging is up. Other photos may be taken of any distinctive trees near the start of the plot or landform features that might facilitate relocating the rebar stake. It may be helpful as well to record the distance and azimuth from the plot origin to the three nearest trees, as well as identifiable characteristics of these trees (e.g., species, DBH). Finally, a quality geo-referenced location with a good GPS instrument must be taken at the rebar stake and recorded. The precision of the GPS should also be recorded—either known horizontal and vertical precision or a general precision estimate (EPE or Accuracy, depending on the specific GPS instrument). We recommend that coordinates be taken with the GPS in the UTM function and set to NAD 83 datum. The UTM zone should also be recorded.

Establishing the belt transect plot. In overview, the sampling plot consists of a transect line (baseline) that is extended from the origin to 150 ft (50 m) in length, thus forming the base of the area to be sampled. The fixed plot is constructed by extending a "belt" out 15 ft (5 m) from both sides of the transect line, perpendicular to the line. Thus, the width of the plot is twice the belt width or 30 ft (10 m). The belt width and the transect length are fixed to create a fixed area plot (4,500 sq. ft or 500 sq. m). (see Figure 1 and Table 2).

The transect line should be oriented along a pre-selected compass bearing and should follow a slope contour (i.e., perpendicular to the slope of the stand). The transect line itself should change less than 50 ft (15 m) in elevation along its length and not shift much in aspect or slope (e.g., should not cross aspects more than 20^0 from origin). To begin, a compass heading can be determined for the transect line by either sighting on a tree or other easily identified landmark. One person walks the transect line out towards the landmark with compass or GPS in one hand and the transect tape in the other, while a second person anchors the transect line zero point at the decided start point. After the transect line has been placed, both ends should be secured with rebar stakes, transect pins, or two surveyor's flags. The monumenting tasks at the start point may be executed after the line is in place.
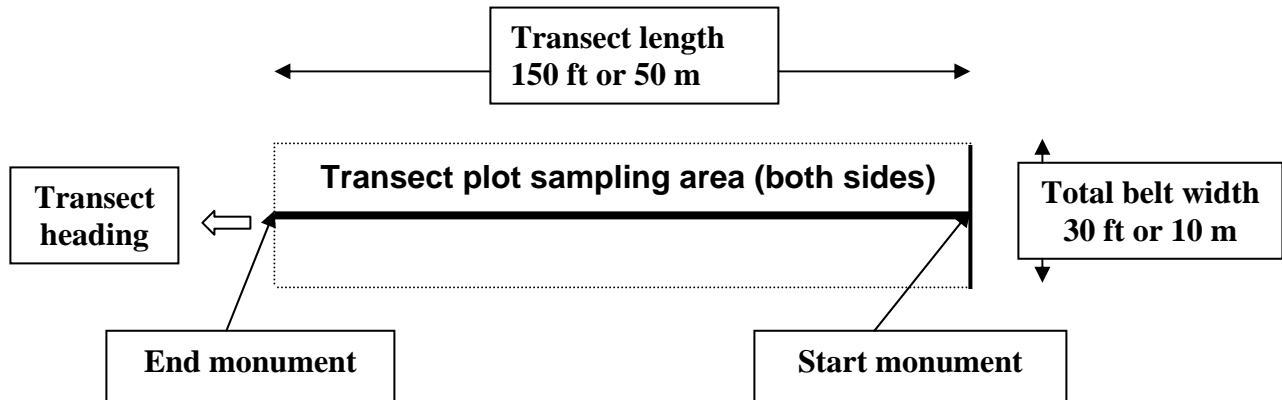
**Transect length
150 ft or 50 m**

Transect plot sampling area (both sides)

**Transect
heading**

**Total belt width
30 ft or 10 m**

End monument

Start monument

Fig. 1. Diagram of transect plot dimensions.

__Whitebark pine composition within the belt transect plot.__ For these methods, the belt transect plot must include living whitebark pine trees greater than 4.5 ft (1.4 m) (DBH) in height and recently dead trees, also greater than DBH height, with foliage still present. Live trees are here defined as those with at least one branch bearing green foliage, and thus may include trees with severe damage from blister rust or other pathogens or insects.

Initial reconnaissance of the sample location should confirm that the selected area within the stand is large enough to accommodate the entire belt transect plot. This is by far the preferred situation. However, if the stand is small and patchy, such as typically encountered in the central and northern Canadian Rocky Mountains, and the size will not accommodate a 150 ft (50 m) transect line, a second transect line may be placed parallel to the first transect by doubling back, with a 9 to 15 ft (3 to 5 m) gap in between plots, denoting the transects as "a" and "b." The two transects do not need to be of equal lengths, as long as they equal a total of 150 ft (50 m). Both ends of both transects must be monumented, although data are treated as if for a single plot. If the trees are predominantly old snags that were killed by mountain pine beetle, the value of plot data from that stand may be questionable.

Note: These methods should be regarded as guidelines; all situations are difficult to anticipate, and field crews may need to make some decisions about how to proceed under different circumstances. With respect to relevé sampling, the guiding principle is *representativeness*, and thus judgments must be made about the appropriateness of unusual conditions for sampling.

Once the transect baseline is established, two people can move along the length of the transect line, setting up the plot. One person at the transect line anchors the measuring tape on the transect line, and the other extends the tape to 15 ft (5 m) and places a wire stem flag or ties surveyors tape to a tree branch every 10 ft (3 m) at the belt width end.   Belt flagging can be more or less frequent than every 10 ft (3 m), depending on visibility from flag to flag.   With three people, the process goes even faster: One person stands at the center of the belt (e.g., mid-length of belt, 15 ft (5 m) over the transect line, and the other two hold the 0 end and total belt width end 30 ft (10 m), respectively, placing surveyors flags or tape again every 5 to 10ft (2 to 3 m). Flagging creates a boundary to the sampling area, which speeds up sampling. Inventory of rust incidence and damage commences once the entire transect plot is flagged. Where ground flags are not clearly visible because of dense understory, the field crew should tie surveyor's

tape to stems or branches, marking the boundary. Flagging to mark the belt boundary should be done as the belt is established. In addition, trees borderline at the belt width boundary should be identified during the plot set-up phase with flagging to show which are in and which are out. **Note:** <u>For the purposes of these methods, a tree is considered "inside" if the center of the base of the tree (i.e., half or more of the tree base) is inside the plot.</u>   This means that a stem rooted inside the plot but leaning outside should be counted as "in."

Summary of belt transect plot set-up steps

- Select stand for sampling; select plot location
- Reconnoiter selected location for suitability
- Establish line transect to 150 ft (50 m)
- Monument starting point: description, rebar and tag, photos, GPS data
- Establish plot by flagging at belt width
- Monument end point with rebar stake, numbered tag, photos, GPS data

Table 2. Key belt transect plot variables
Initial length: 150 (50 m)   **Belt width: 15 ft (5 m) on each side**   **Total width: 30 ft (10 m)**   **Final area sampled for trees > 4.5 ft (1.4 m) in height: 30 ft x 150 feet = 4,500 ft^2 (10 m x 50 m = 500 m^2)**   **Area sampled for understory survey: 30 ft x 150 ft = 4,500 ft^2 (10 m x 50 m = 500 m^2)**   <u>**Note**</u>**: Do not mix measurement systems: stay with English (Imperial) or metric**

Transect description. The following general information must be recorded on the data sheet to describe each transect plot. As noted previously (see **Personnel and training requirements**), we assume that field personnel (or at least the crew leader) are experienced in collecting the basic descriptive information that follows.
- **Plot number (a unique number for each plot)**
- **Date:** 2 digits each for month and day; 4 digits for year (e.g., 06202004 is June 20,2004).
- **Start (origin) monument tag number:** 3 digits, matches the metal tag on the stake.
- **End (terminal) monument tag number:** 3 digits, matches the metal tag on the stake.
- **Field team:** names of each member, with leader named first.
- **State or province:** two-letter code.
- **Administrative unit:** name of National Forest and District, Wilderness Area, National Park, or private landowner and address. This can be obtained from USGS topographic maps, national forest, or park maps.
- **Specific location**: drainage, trailhead, and estimated distance from start of trailhead or other landmark. Topographic maps are best used for reference.
- **Topographic map ID:** give the appropriate map name and/or number.
- **Units of measurement**: metric (m, cm) or English (Imperial) (ft, in). Once this is decided, all measurements must be in the same system. Most scientific studies today

use the metric system. However, since some field personnel are not comfortable with this system, English units are acceptable. Plot areas can easily be interconverted.

- **Compass direction of transect line:** This is recorded at the *starting point* and then again at the *center* of the line in degrees using a compass or GPS.
- **Final transect length**: In feet or meters recorded to the nearest entire foot or meter. This length may vary if a transect is broken into two or more shorter lines, e.g. as in patchy stands. Alternatively, some field teams may chose to extend or shorten transect lengths depending on their surveying objectives.
- **Aspect (azimuth)** of sample stand **at center of transect line:** Measured with a compass or GPS unit to the nearest 10^0.
- **Elevation at center of transect line (ft or m)**: Elevation estimates may be obtained from a GPS unit. If the satellite signal is insufficient, estimates may be made from a topographic map.
- **Slope steepness at transect line center:** The slope should be estimated to the nearest % or 5^0 with a clinometer.
- **GPS location**: This involves recording a UTM easting and northing for the beginning and endpoint of transect line, using a GPS with 10 m accuracy or better.   In addition, the UTM zone should be recorded as well as the map datum used for location [e.g., NAD83 (recommended) or NAD29]. It is also useful to record the precision of the GPS reading, which will help guide future field teams relocating the monuments.
- **Successional status:** Classify the successional status of this sample stand using the following codes:
  C – Climax or near climax. Site is occupied by tree species that will not change over time in the absence of disturbance.
  L - Late seral. Stand is composed of an overstory of seral and climax species with an understory of climax species.
  M – Mid-seral. Stand is composed of mostly seral species in the overstory.
  E – Early-seral. Stand is composed of shade-intolerant shrubs, herbs, or trees seedlings and saplings.
- **Habitat type or cover type:** Key the habitat type from the habitat type classification most appropriate for your sampling area. Indicate whether known from previous surveys, GIS overlays, or estimated by consulting with habitat type manuals; if the latter, name the reference.

Note: Crew should not spend much time determining the latter two categories. Some information can be added later with the aid of reference sources.

- **Estimated proportion of each tree species in overstory:** Estimate relative proportions of each species of cone-bearing trees, especially of whitebark pine, in the forest canopy—defined here as over 15 ft (5 m) in height. Consider the canopy cover that is present to be equal to 100% (even if part of the plot has no canopy cover). The percentages of the tree species comprising the canopy cover should add up to 100%.
- **Woody and herbaceous plant dominants:** Name three to five of the most commonly-occurring plants in the undergrowth, both woody and herbaceous. (If necessary, specimens may be collected for later identification.)
- **Rust-resistant candidate trees**: This applies only to stands where blister rust has together infected and killed more than 90% of the trees above 4.5 ft (1.4 m) in height. At the completion of a plot with this level of blister rust damage, any trees that appear to be free of blister rust symptoms should be tagged and recorded as potentially rust-resistant trees for future seed collections with notes made on the data sheet including GPS position.

Sampling within the belt transect plot

Which trees count. The field crew must start at the zero end of the plot and work back and forth across the entire belt width, tagging and examining each tree in the order of nearest to farthest from the starting rebar. Tag numbers increase accordingly.   For each dead or live whitebark pine encountered that is <u>greater</u> than 4.5 ft (1.4 m) in height, the crew records diameter at breast height (DBH) in either inches or centimeters using a DBH tape (keeping the measurement system consistent with transect and plot establishment) *on the uphill side of the tree*. Fallen snags that are still rooted but leaning are counted as dead trees if at greater or equal to a 45° angle to the ground. **Note:** <u>For a tree to be included within the belt transect, at least half of the tree base must be within the plot boundary.</u>

 If a tree forks and the stems are separate below breast height, each stem is measured and evaluated separately. If a "trunk" splits off the main bole below DBH but clearly above the root/base, it should be considered a branch of the tree and not a separate stem. These branches often curve outwards and upwards. The same rule applies to groups of stems: Stems that are separate below breast height, although they occur within a group or "tree clump," are each evaluated. Stems whose bases occur outside the plot are not included; it is thus possible to have some stems of a clump counted and others not. On the data sheet, stems that are connected either by fusion or by growing contiguously in the same "tree clump" should be designated with a letter [e.g., tree # 1 is a single tree (tag #220) so it would not have a clump letter assigned to it; the second tree has 4 stems, so the stems would be counted as trees #2 to #5 and labeled 221a, 222b, 223c, 224d on the data sheet; and the next tree #6 is not a clump, so it would be tagged and labeled 225]. Thus, all labeled stems count towards the total trees for the plot. In review, stems that are outside the plot boundary are neither tagged nor examined. Only stems that are taller than breast height are included in this survey.

In krummholz growth form situations, where adult trees are < 4.5 ft (1.4 m) tall, most of the trees appear to consist of stem clumps. Ideally each stem should count as a tree and be assigned clump numbers and letters as appropriate. In dense krummholz trees, however, stems may be very difficult to trace to the tree base. In the interests of time under these circumstances, each dense clump may be tagged as a single tree and examined as thoroughly as possible.

We reiterate that crews should measure and tag trees in the order of their occurrence as one traverses from the beginning to the end of the belt transect plot, which facilitates locating and remeasuring the same trees over time. So, the first trees counted are the ones that are the closest to the baseline of the transect plot. As previously described, all surveyed whitebark pine trees above DBH height must be tagged with aluminum tree tags (available from any forestry supplier) in order of measurement. We recommend that these trees are tagged at breast height level facing the starting rebar monument, for ease of relocation. Trees that are barely above breast height may be tagged farther down the stem where the diameter is wide enough to accommodate a nailed tag, and a note should be made to that effect on the data sheet.

Tree assessment: living and dead. Each living whitebark pine taller than breast height is examined for blister rust symptoms, canopy kill, bark stripping, and mountain pine beetle infestation. Each dead tree is assigned a cause of death, if detectable. The following information, summarized in Table 3, is recorded on the data sheet:

- **Tag number**
- **Stem clump letter:** If stems are separate below breast height, each stem is tagged and receives a letter designation to indicate clump membership.
- **Tree status**: (H) Healthy, (S) Sick, (R) Recently dead, and (D) Dead snag. For living trees, this field should be filled in only after tree assessment below is completed. Trees rated as "Healthy" trees have no apparent active or inactive cankers or pine beetle infestation. Any dead branches or bark stripping is confined to a small portion of the tree (e.g., < 10%) and could result from mechanical damage. In other words, there are no symptoms to indicate that the tree actually or potentially has blister rust or hosts mountain pine beetles. For mature trees, the H category signals the potential for cone production in the near future. If a tree has cankers, > 10% branch kill, heavy bark stripping, or pine beetle infestation, it should be categorized as "sick." Recently dead trees still have brown foliage present, and snags are older dead trees with no foliage remaining. A tree is considered living if it has even one branch with green foliage.
- **DBH**: Diameter is measured at breast height for all trees greater than 4.5 ft (1.4 m) in height to the nearest 1/10 inch (2.5 mm).

 For living trees:
- **Stem (trunk) cankers:** The stems of trees are searched with special care to locate potentially active cankers (sporulating with new or old aecia, i.e. spore sacs), or inactive cankers. For reference pictures of active and sporulating cankers, see Hoff (1992) and Hunt and Meagher (1992). For large trees, trunks should be searched with the aid of binoculars from at least three different positions. For moderate to large trees, this is most efficiently accomplished with 2 to 3 people examining different sides of the tree at the same time. The task becomes easier with experience. Each person should spend about 2 to 3 min carefully surveying a given trunk for cankers. Small trees should be searched carefully by moving foliage and branches. As soon as one active stem canker is found, the search may be ended. **Note:** Extensive training is required to identify cankers. Cankers are recorded as Active (A), Inactive (I), Uncertain (U), None (N), and Other (O). "Other" refers to a suspicious but not definitive canker-like symptom.   "Uncertain" occurs in circumstances such as heavy lichen cover where bark is not visible. **Note:** Trees with only "Uncertain" cankers should not be included in the final data analysis. Because stem cankers are almost always fatal, this information enables projection of certain mortality in the stand. Only the "highest" order of canker activity should be recorded (i.e., if there are active cankers as well as inactive cankers, then A should be recorded).
- **Branch cankers:** We consider the tree canopy to include all main branches that begin as bifurcations off the trunk and to encompass all foliage and supporting twigs and side-branches. The canopies of trees should be searched with care to locate potentially active branch cankers (sporulating: with new or old aecia spore sacs), or inactive cankers. For large trees, canopies should be searched with the aid of binoculars from at least three different positions. Again, the most efficient approach, whenever possible, is to have 2 to 3 people search different sides of the canopy of moderately large to large trees simultaneously. In the interest of time and efficiency, only 2 to 3 min should be spent per position surveying the canopy for branch cankers. (Previous efforts have shown that all branch cankers cannot be detected in tall trees with large canopies; the smaller ones are particularly hard to see.) Small trees should be searched carefully by moving foliage and branches. The presence of newly dead branches or branch tips with red-brown foliage (branch "flagging") often indicates the presence of an active canker, but not with certainty. Dead foliage may also result from other causes, including drought and insects.

As soon as one active branch canker is found, the search may be ended. **Note:** <u>Training is required to identify cankers</u>. Cankers are recorded as Active (A), Inactive (I), Uncertain (U), None (N), Other (O). "Other" refers to a suspicious but not definitive canker-like symptom. "Uncertain" occurs in circumstances such as heavy lichen cover, where branches cannot be clearly observed. **Note:** *Trees with only "Uncertain" cankers should not be included in the final data analysis.* <u>The "highest" order of canker activity should be recorded (i.e., if there are active cankers as well as inactive cankers, then A should be recorded).</u>

- **Canopy kill (topkill)**: As noted above, the canopy includes all main branches that begin as bifurcations off the trunk and encompasses all foliage and supporting twigs and side-branches. The percentage of the total canopy that has been killed (dead branches) is estimated by assigning each tree to one of the following classes: 1 (0-5%), 2 (6-15%), 3 (16-25%), 4 (26-35%), 5 (36-45%), 6 (46-55%), 7 (56-65%), 8 (66-75%), 9 (76-85%), 10 (86-95%), 11 (96-100%). The entire canopy volume is visualized in the process of making this estimate. The percentage killed is considered as a portion of this volume. Branches may be dead for only a portion of their length. All dead branches are considered, whether caused by blister rust, bark stripping, or mechanical damage, which are often difficult to distinguish.

- **Presence of bark stripping**: Squirrels and porcupines commonly climb trees to eat cambium, but squirrels are particularly attracted to active blister rust cankers for the sugars produced. The stripped condition of branches may range from short sections of bark removed to the entire length of branches stripped bare. The level of bark stripped on a tree should be noted as (N) None, (L) Light, (M) Moderate, and (H) Heavy stripping. Categorizing trees is somewhat subjective; however, the following guidelines are based on numbers of branches affected rather than total area of bark stripped: L is defined as fewer than 10% of total branch area in the canopy volume showing some degree of stripping, M refers to greater than 10% but fewer than 20% of the branches stripped, and H refers to more than 20% of the canopy showing bark stripping to any extent, or the stem of a small tree partly or completely stripped.

- **Presence/absence of mountain pine beetle**: Mountain pine beetle infestation is another major mortality factor in whitebark pine. Either beetle entry holes with pitch plugs or J-shaped galleries in the wood indicate their presence in a tree. Record presence with a check mark in the appropriate column on the data sheet. Removal of a section of stem bark will be necessary to view beetle galleries.

For dead trees:

- **Cause of death in trees:** Blister rust cankers and the galleries of mountain pine beetles are often still evident in recently dead trees. Descriptors for the cause of death are Blister rust (R), Mountain pine beetle (B), or Other/unknown (U). During the last few field seasons, we have been seeing dead and dying trees simultaneously hosting both blister rust and mountain pine beetles, although pine beetles are likely the ultimate cause of death. In addition, several years of drought may be taking a toll. Notes may be made on data sheets in order to elaborate on any tree condition.

Table 3. Data recorded for each tree	
Tag number	Status of tree: healthy, sick, recently dead, dead snag
DBH (living and dead trees)	Stem clump letter designation (living and dead trees)
Stem cankers: active, inactive, uncertain, none, or other (living trees)	Bark stripping: none, light, moderate, heavy (living trees)
Branch cankers: active, inactive, uncertain, none, or other (living trees)	Presence/absence mountain pine beetle (living trees)
Percent canopy kill class: 1-11 (living trees)	Cause of death: blister rust, mountain pine beetle, or other/unknown

Tree Understory survey. Stands vary greatly in the amount of understory tree growth present, especially with successional status and successional stage. However, it is important to know to what extent whitebark pine regeneration, whether suppressed or healthy, is infected by blister rust in order to anticipate stand health in the future. This survey is undertaken for the basic belt transect plot that is 150 ft x 30 ft (50 m x 10 m) as required information. It is designed to be completed quickly.

Definition of understory: For purposes here, we define understory as all trees that are up to and including 4.5 ft (1.4 m) in height. For expediency, the understory is divided into stems that are less than or equal to 20 in (50 cm) in height and stems that are greater than 20 in (50 cm) and up to 4.5 ft (1.4 m) in height.

Completing the understory survey.   We recommend that field crew members place a piece of masking tape at 20 in (50 cm) on one pant leg and another at breast height. Each understory stem or clump may be rapidly categorized into a size class and evaluated by this method ("cruising"). To avoid resampling the same stem twice, each counted stem may be marked with a wire stem flag. For this survey, all stems that are forked or in stem groups are counted as one stem, that is--a single regeneration "site." If only one stem of a clump is above 20 in (50 cm) in height, then the entire clump is categorized at that height. If only one branch of a fork or one stem of a group bears a canker, than the entire regeneration site is considered infected. Only living stems are included in this survey. For the understory stems or stem clumps, cankers are categorized as previously described for overstory trees: active canker, inactive canker, no canker, or "other." (The "uncertain" category is eliminated.) The most severe category of canker is reported for each single stem or clump. For example, if a seedling has an inactive and an active canker, only the active canker is recorded. Cankers are not separated into branch and stem cankers. Stems are tallied by height and canker categories with dots or tick marks during the survey, and counted up for each category at the end (Table 4). In krummholz situations, where all trees may be < 4.5 ft (1.4 m), the whitebark pine understory should be upright, growing seedlings.

Table 4. Understory survey data sheet sample				
Height category	**Active canker**	**Inactive canker**	**No canker**	**Other**
≤ 20 in (50 cm)				
> 20 in (50 cm)				

IV. Basic Descriptive Statistics and Data Analysis

After data are entered in the database program, the following data summaries may be calculated for each belt transect plot. The data summaries below correspond to queries that are incorpored into the Microsoft Access database, *Whitebark Pine Blister Rust Survey: Database Application,* Beta Test Version 0.20, designed specifically for these methods by David Pillmore and Brent Frakes (Rocky Mountain Network Inventory and Monitoring, USDI National Park Service, Ft. Collins, Colorado). **Note:** <u>Living trees with only "Uncertain" cankers should not be included in the database because of underestimates of percentage infected trees</u>.

Trees

For trees that are greater than 4.5 ft (1.4 m) in height, the following basic **summary statistics** may be calculated:
- Number of trees
- Plot area
- Density of trees
- Percentage of live trees with active blister rust cankers, both branch and stem
- Percentage of live trees with active stem cankers
- Percentage of live trees with any history of cankers (active, inactive, and other)
- Percentage of live trees with canopy kill
- Average percent category of canopy kill
- Percentage of live trees with bark stripping
- Percentage of live trees with bark stripping definitely associated with blister rust (with active and inactive cankers)
- Percentage of live trees with mountain pine beetle, both early and advanced stages
- Percentage of trees sampled that are dead
- Percentage of dead trees with symptoms of blister rust
- Percentage of dead trees with symptoms of mountain pine beetle
- Percentage of dead trees with unknown or other cause of death

Additional information of interest for trees greater than 4.5 ft (1.4 m) in height.

Trees may be divided into the following diameter class categories: 0 – 4.9 in, 5 – 9.9 in, 10 – 14.9 in, 15 – 19.9 in, 20 – 24.5 in, > 25 in (0 – 12.5 cm, 12.6 – 25.1 cm, 25.2 – 37.8 cm, 37.9 – 50.5 cm, 50.6 – 62.2 cm, > 62.3 cm).

Percentage of trees vs. different diameter class categories:

- Infected by blister rust
- With canopy kill
- With bark stripping associated with blister rust
- With mountain pine beetle infestation
- Dead
- Dead with symptoms of blister rust

Understory survey:

For trees that are 4.5 ft (1.4 m) and under in height, the following basic summary statistics may be calculated:

For all stems/clumps combined and also for stems/clumps divided into > 20 in (50 cm) and ≤ 20 in (50 cm) in height:
- Density of understory stems or clumps
- Percentage of stems/clumps with active blister rust cankers
- Percentage of stems/clumps with any canker signs (active, inactive, and other)

V. Add-on Methods for Gathering Additional Data

For more complete but more time-consuming surveys, there are methods "add-ons" that we recommend. These are useful for targeted research or for obtaining a more detailed picture of either the habitat type or dynamics of spread of blister rust.

Counting cankers
The purpose of this add-on is to examine the infection intensity within a stand. This enables us to differentiate highly infected stands from lightly infected stands, and to track changes within a stand and among stands through time. This is probably the most important add-on but also the most time-consuming, given the great effort and time required to search large trees for multiple cankers. In this situation, every branch and stem must be examined thoroughly, either by distant inspection aided by binoculars or by climbing trees for close inspection. The important variables for data collection include:
- **Number of active branch cankers**
- **Number of active stem cankers**
- **Number of inactive branch cankers**
- **Number of inactive stem cankers**
- **Number of "other" branch cankers**
- **Number of "other" stem cankers**

Ribes survey

This add-on is for the purpose of correlating blister rust infection percentages or intensity within a stand with the density of *Ribes* shrubs or the presence of certain Ribes species in the understory. **Note:** <u>The value of simply counting the shrubs present along a transect without keying out (identifying) shrub species was considered questionable by pathologists.</u> Possible variables for data collection:

- **Presence/absence of *Ribes* in understory**
- **Number of *Ribes* stems in understory**: These data enable a density calculation for the transect.
- **List of *Ribes* species found on the transect**
- **Number of *Ribes* stems per species found on the belt transect plot**: This information permits a density calculation for each species that occurs on the belt transect plot.

Potential regeneration survey

This add-on focuses on actual healthy seedlings and saplings in the understory and excludes suppressed (degenerate) regeneration. Possible guidelines for including only growing seedlings include:

- **Stems with clear apical dominance**
- **Stems with recent growth of about 1 in (2.5 cm)**
- **Stems that are straight and have foliage for more than 50% of their length**

Other forest trees

Including all forest trees in addition to whitebark pine produces a more complete picture of habitat type, successional stage, and overall forest tree density, which may affect spore transmission distances and micro-climate. In the interests of time, it is recommended that only trees greater than 4.5 ft (1.4 m) be included in the survey.
- **Tree identification:** species
- **Tree DBH**

Whitebark pine ages

Because of differences in radial growth rates with climate and topography, it may be useful to age a subset of whitebark pine trees within a belt transect plot in order to examine stand age structure and the relationship between blister rust susceptibility and age. This requires the use of an increment core, preparation of cores for ring counts, and accurate counts that are paired with DBH measurements. It is recommended that increment cores be obtained from the tree base rather than breast height for the most accurate ages. We recommend that cores be taken from across DBH classes to be meaningful, and that several cores be obtained per DBH class.

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Appendix I

Blister rust survey methods reviewed

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Appendix II

Sample Date Sheet
Summary Field Sheet
(Attached)

Blister Rust Survey and Monitoring Data Sheet
Belt Transect Plot Description and Understory Survey

Plot No: _____   Start Monument Tag #: _ _ _   End Monument Tag #: _ _ _

Date (mm/dd/yyyy): _ _ _ _ _ _ _ _   Field Team: _____

State/Province (2-letter code): _ _   Administrative Unit: _____

Specific location: _____

Units of measurement (check): Metric ( ) English ( )   Topo Map ID: _____

Type: Transect ( ) Circle ( ) Rectangle ( )   Length (nearest 1.0 m or 1.0 ft): _____

Center of plot: Elev: _____ m ft (circle one) Slope: ___ % deg (circle one) Aspect (to 10°): _____

Start GPS: NAD: _ _ Zone: _ _ Easting/Long: _____ Northing/Lat: _____ Accuracy: __

End GPS: NAD: _ _ Zone: _ _ Easting/Long: _____ Northing/Lat: _____ Accuracy: __

Compass direction of transect (True North):   at Start: _____   at Center: _____

Successional status (C, L, M, E): _____

Habitat type: _____   Cover type: _____

Reference for above: _____

Estimated percent of each tree species in overstory: _____

_____

Undergrowth dominants: _____

_____

Photo info. (roll/number):   Along transect from origin: _____   End of right belt: _____

Along transect toward origin: _____   End of left belt: _____   Other:_____

Rust resistant candidate trees (plus trees), tag # and GPS location: _____

_____

Comments (cone production, nutcracker activity, etc.): _____

_____
_____
_____
_____

UNDERSTORY SURVEY: trees < DBH 4.5 ft (1.4 m)
Complete this tick mark matrix for all LIVE understory whitebark pine within the belt transect.

Height < DBH	Active Cankers	Inactive Cankers	No Cankers	Other
≤ 50 cm (20 in)				
> 50 cm (20 in)				

Plot No.:   **Date:**

Tree No.	Tag # or Dist Along/From Tape	Clump letter a,b,…	DBH	Stem Cankers A,I,N,U,O	Branch Cankers A,I,N,U,O	Canopy Kill % class	Bark Strip N,L,M,H	MPB Pres. √	Tree status H,S,R,D	Cause of Death R,B,U	Notes
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Cankers: A=active (spores), I=inactive, N=none, U=uncertain, O=other; Tree status: H=healthy, S=sick, R=recently dead, D=dead
Canopy kill classes: 1(0-5), 2(6-15), 3(16-25), 4(26-35), 5(36-45), 6(46-55), 7(56-65), 8(66-75), 9(76-85), 10(86-95), 11(96-100)
Bark stripping: N=none, L=light, M = moderate, H= heavy; Cause of tree death: R=rust, B=beetle, U=unknown/other