

2009 *Bridgeoporus nobilissimus* Genetic Presence in *Abies* Trees

Survey Report

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 BLM Contract L09PX01364
 Dec. 29, 2009

This report summarizes the methods and results of a survey for the presence of the fungus *Bridgeoporus nobilissimus* in stands containing known sites of this fungus. *B. nobilissimus* forms perennial conks on large-diameter noble fir (*Abies procera*) and silver fir (*Abies amabilis*) stumps, snags, and live trees, but conks are extremely rare. Using a specific genetic marker for *B. nobilissimus* and DNA extracted from wood cores, it was shown in a previous study that some trees near conk-bearing trees contain *B. nobilissimus*, although they do not support a conk and show no outward signs of infection (Gordon 2008). The purpose of this study was to survey over larger areas for the presence of *B. nobilissimus* in stands surrounding known sites of the conk.

Six stands were surveyed between July and September of 2009. The survey area information is given in Table 1. Fixed plots were sampled surrounding all known sites for 1/24 acre up to 1 acre depending on the number of true fir trees in the immediate vicinity. Four of the survey areas were given extensive monitoring in which 10 to 17 variable plots were installed using a basal area factor (BAF) based upon the surrounding stand and individual tree diameters. The area included in each of these four survey areas varied from about 15 to 35 ha. Two of the survey areas were sampled only in the immediate vicinity of the *B. nobilissimus* conk.

Table 1

Site Name	Administrative Unit	Survey Type	Number of Known Sites (conks)
Mary's Peak	Siuslaw N.F., Oregon	Extensive	1
Snow Peak	Salem B.L.M., Oregon	Extensive	1
Gordon Meadows	Willamette N.F., Oregon	Extensive	2
Mt. Rainier	Mt. Rainier N.P., Washington	Known site only	1
Wildcat Mtn.	Mt. Hood N.F., Oregon	Extensive	3
Asahel Curtis	Mount Baker- Snoqualmie N.F., Washington	Known site only	1

A sampling protocol was developed by agency statisticians (Appendix A). Sampling of each tree consisted of using a disinfected increment borer to remove 4 wood cores, approximately equally spaced around the circumference at the base of the tree. For highly decayed trees from which cores could not be obtained, 4 small pieces of rotten wood were removed from different points on the stump. Every sampled tree was tagged with a metal tree tag with a unique number. Each set of cores was bagged and labeled to identify the plot and tree number from which the sample was taken. Data that was taken in the field included tree location, whether it was a tree or a stump, live or dead, the species (only *A. procera*, *A. amabilis*, and unknown *Abies* were sampled), the diameter at breast height, the decay class for dead trees, the site, the BAF, and the tree tag number.

Samples were kept in coolers with ice until they reached the lab, where they were refrigerated. In the lab, the samples were processed according to methods developed and tested previously (Gordon 2009). Briefly, the contents of each sample bag were ground in a disinfected blender, a subsample of the ground wood was removed and the total DNA was extracted from this sample. The raw DNA was cleaned using a commercially available kit, and the cleaned DNA samples were run in a PCR with the *B. nobilissimus*-specific primers. The PCR products were run in a gel containing a DNA dye, and the presence of a 550 base pair product was used as an indicator of the presence of *B. nobilissimus* DNA in a sample.

The results of this testing were correlated with the field sample data and saved in a spreadsheet. The data will be analyzed by agency biostatisticians. Generally, the fungus was found to be widely distributed in the stands sampled. Although the six survey areas contained only 9 conks, 91 trees (out of 425 tested) were found to contain the fungus. Sixty-eight of the positive trees sampled were alive rather than snags or stumps. Five of the positive trees were *A. amabilis* and 4 of those trees were from the two Washington sites. At Mary's Peak and Snow Peak, *B. nobilissimus* was found in trees over 500 m from the nearest conk. Although it has been reported that conks are only found on large diameter trees and stumps, in this study *B. nobilissimus* was found in trees as small as 3.7" in diameter.

References

Gordon, M. 2009. Detecting *Bridgeoporus nobilissimus* in wood cores using a genetic test: a test of a core processing method and method sensitivity. Final report submitted to USDI Bureau of Land Management, Oregon State Office. On file with: Interagency Special Status/Sensitive Species Program, USDI Bureau of Land Management, Oregon State Office.
<http://www.fs.fed.us/r6/sfpnw/issssp/documents/inventories/inv-rpt-fu-brno-woodcoreprocessingmethods-2009.pdf>

Gordon, M. 2008. Field methods for the detection of *Bridgeoporus nobilissimus* DNA in trees, stumps, and snags. Final report submitted to USDI Bureau of Land Management, Oregon State Office. Contract number HAP084563. On file with: Interagency Special Status/Sensitive Species Program, USDI Bureau of Land Management, Oregon State Office, Portland, Oregon; USDA Region 6 Forest Service, Regional Office, Portland, Oregon.
<http://www.fs.fed.us/r6/sfpnw/issssp/documents/inventories/inv-rpt-fu-brno-field-methods-fordetection-2008-12-09.pdf>

Appendix A

Survey Protocol for Testing *Bridgeoporus nobilissimus* Detectability at the Site Level

Version 8/20/2009

Preparers: Kelli Van Norman, Carol Apple

Introduction

The presence of the fungus *Bridgeoporus nobilissimus* (BRNO) is known from locations where it produces a conk on dead or live boles and stumps of noble fir (and Pacific silver fir at the one Olympic NF site). Preliminary samples taken from live and dead trees near known conk sites have confirmed BRNO presence.

Objectives

The objective is to estimate how many true fir trees in a population have the BRNO fungus. A secondary objective is to get an idea of the extent of the fungus.

Methods

Office Work

Within an area where a known site is located (i.e., BRNO presence confirmed), select a homogenous forest stand of suitable habitat around the known site that can be spatially delineated. Create and save a GIS polygon of the selected area. Calculate the acres.

Identify if any known positive trees or stumps are in the area and determine their locations (GPS location?). If there are directions to the known sites, make a copy of the directions to take out in the field. A 1/24 ac (24 ft. radius) fixed plot will be established around those trees/stumps, these plots will be considered a separate stratum.

Stratification can also be used for other homogenous areas within the population of interest (the acre sizes do not have to be the same). For each stratum, delineate a GIS polygon and calculate the number of acres.

Make a map to print on 8 ½ x 11 paper showing the delineated stand and the known site location(s) overlaid on an air photo and include the map scale.

Select 10 random points in the homogenous delineated area. The points can be placed using a systematic sample with a random start. Be sure to use a random start, it is important for the analysis. If more than one stratum is delineated, take 10 sample points in each stratum. The points can be placed on the map prior to going to the field or determined when at the site. These points should not fall in the fixed plot area of the positive known sites.

The determination of the basal area factor (BAF) will be determined in the field.

If after the lab work it is found that there were no positive hits, then select additional random points and resample. We acknowledge that this is a form of sampling where the use of a previous sample is used to determine if more samples are needed and that will be taken into consideration.

Field Work

Fixed plot for known positive tree/stump

Locate each previously known positive tree/stump in the area. At each location establish a 1/24 acre circular plot (24 ft. radius), placing the plot center a short distance away from the North side of the tree/stump. If a 1/24 area plot will not pick up enough trees/stumps (minimum number of trees/stumps should be 5-7), you have the flexibility to use a larger radius. Be sure to record the radius used. Place a stake at the plot center. These fixed plot areas are considered a different stratum.

Include only noble fir and Pacific silver fir species of live trees with a minimum dbh of 5 inches within the 24 ft. radius plot. If a larger plot is used, include live trees with a minimum dbh of 10 inches for the plot area outside the 24 ft. radius. All snags will be measured on the plot; there is no lower dbh limit. For stumps within a plot radius of 16.65 ft., include those with a diameter of 5 inches or greater. Between 16.65 ft. and 24 ft. plot radius, only include stumps with a diameter greater than 20 inches. Stumps are the portion of a tree remaining after the trunk has been severed at a point less than 4.5 ft. above ground on the uphill side. Label each tree/stump with a tag attaching it 3 to 6 inches above the ground and facing the plot center. Be sure to leave at least 1 ½ inches of the aluminum nail sticking out of the tree to allow for growth.

Record the tag number, tree dbh or stump diameter, live/dead tree status, species, decay class for snags and stumps (see table attached), and the azimuth and distance from the plot center. The distance is for relocating the tree/stump so measure distance to the face of the tree/stump from plot center.

Bore all the tagged trees and stumps in the plot using an increment borer. The boring will be done on 4 sides of the tree/stump (downhill, uphill, and sides) at approximately 1.6 ft. (0.5 m) above ground. When starting to bore, position the auger to maximize the depth that can be achieved. It is sometimes beneficial to angle the auger slightly downward, but too much of an angle prevents the full depth from being reached because the tree trunk eventually prevents the handle from rotating. If a stump is short, it can be cored from the top angled in and downward; just be certain to get four representative samples. All wood samples from one tree can be mixed into one labeled sample bag (e.g., recloseable plastic bag). Label the bag with an abbreviation for the site, the fixed plot "F", the plot number and tree number (Mary's Peak, fixed, plot 1, tree 5 would be labeled as MP-F1-5). If the ambient temperature is over 50 ° F (10° C), place the sample bag in a cooler.

To prevent cross-contamination, clean the increment borer or drill bit after each tree and stump. The cleaning method is as follows (Gordon 2008): First the auger and quill extractor are agitated in a wash solution, a 1% aqueous solution of the surfactant Triton X-100 to remove the shreds of bark that tend to cling to the outside of the auger. Then the auger and extractor are placed in disinfecting solution, a 1% solution of Triton X-100 in isopropyl alcohol. This is the disinfection step. Allow the auger to soak in the disinfection solution while you insert a fresh cleaning patch into the slotted end of the gun cleaning tool. Remove the auger and allow it to drain back into the bottle. The extractor is then wiped with a clean dry cloth, and the inner surface of the auger is cleaned with a .22 rifle cleaning tool. This tool consists of a rod with a slotted end. A small fabric cleaning patch is inserted in the slot, and the rod is pushed up and down inside the auger. A new cleaning patch must be used for each cleaning. Finally the outside of the auger is dried with a clean dry cloth. The cleaning solutions can be kept in 1 liter bottles with tight-fitting tops so they could easily be transported in a backpack or bucket to sampling sites. Follow a similar method for disinfecting drill bits if they are used.

Spraying WD-40 inside the increment borer after disinfection will greatly reduce the chances of the next core getting stuck in the increment borer and will not harm the wood sample.

Variable plot for trees on unknown sites

The ten random point locations (i.e., sample point) selected during the office work will be the variable plot centers. Locate the first sample point location. Mark the sample point with a wooden stake and take the GPS reading of that location. The sample point should not be within the fixed plot for a known tree or stump since that area is considered a different stratum. If that occurs, randomly select another point to replace it.

At the sample point location, use a wedge prism or Spiegel relascope to determine if a tree is to be included in the sample. To determine the appropriate BAF to use, stand at the plot center and check to see which BAF provides 5 to 7 trees selected as "in". The same BAF must be used within a stratum.

If the tree is borderline, measure the distance from the face of the tree to the plot center to determine if it is "in" (See limiting distance tables). When measuring the distance, correct for slope when slope is greater than 10% (see slope correction factor table). Include only trees with a minimum diameter of 10 inches and noble fir and Pacific silver fir species.

Label each tree with a tag attaching it 3 to 6 inches above the ground and facing the plot center.. Be sure to leave at least 1 ½ inches of the nail sticking out of the tree to allow for growth.

Record the tag number, dbh, live/dead tree status, decay class for dead trees (see table attached), species, and the azimuth and distance from the plot center and bore all the trees that are "in". The distance is for relocating the tree so measure distance to the face of the tree from plot center. The boring will be done on 4 sides of the tree (downhill, uphill, and sides) at approximately 1.6 ft. (.5 m) above ground. Use an increment borer for the boring. See method described above for boring and storage of the samples. Label the bag with an abbreviation for the site, the variable plot "V", the plot number and tree number (Mary's Peak, variable plot 8, tree 2 would be labeled as MP-V8-2).

Clean the increment borer after each tree. Use the method described above.

Fixed plot for stumps on unknown sites

A fixed plot will be used to determine which stumps to bore at the site of the variable plot. Stumps are the portion of a tree remaining after the trunk has been severed at a point less than 4.5 ft. above ground on the uphill side. For stumps within a plot radius of 16.65 ft., include those with a diameter of 5 inches or greater. Between 16.65 ft. and 24 ft. plot radius, only include stumps with a diameter greater than 20 inches. Bore on all four sides of the stumps (downhill, uphill, and sides) within the fixed plot. If a stump is short, it can be cored from the top angled in and downward; just be certain to get four representative samples. Label each stump with a tag attaching it 3 to 6 inches above the ground and facing the plot center. Be sure to leave at least 1 ½ inches of the nail sticking out of the tree to allow for growth.

Record the tag number, species (if possible), cross section, decay class (see table attached), and the azimuth and distance from the plot center. See method described above for boring and storage of the samples. Label the bag with an abbreviation for the site, the variable plot "V"(this

plot is at the variable plot site), the plot number and stump number (Mary's Peak, variable plot 9, stump 5 would be labeled as MP-V9-5).

Clean the increment borer after each stump. Use the method described above.

After Field Work

Samples stored in plastic bags will quickly grow moldy if the temperature is over 50 ° F (10° C). During transportation and storage, the samples should be kept between 32 ° to 41 ° F (0° to 5° C). Wood samples have been stored successfully (*B. nobilissimus* DNA still detectable) for over 1 year under refrigeration. Freezing will preserve the DNA within the sample, but repeated freeze/ thaw cycles will degrade DNA, and should be avoided so store the samples in a refrigerator prior to handing off the samples to the genetics lab. The samples will be given to a contractor for lab analysis to test for BRNO presence.

Equipment List





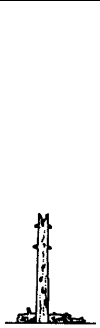
Be sure to have enough equipment for each crew.

- Personal protection equipment including hardhats and gloves
- Field forms
- Survey protocol
- Aerial photo maps with stand delineated
- Clipboards/tatums
- Pencil
- Permanent marker pens
- Wooden stakes
- Flagging
- Calculator
- Compass
- GPS device
- Tree tags
- Hammer
- Aluminum nails
- Measuring tape (50 foot and 100 foot)
- D-tape or logger's tape in English units
- Spiegel relascope or Wedge prisms of BAF 20, 40 and 60
- 16-20" increment borers with .25" internal diameter (A smaller increment borer can be used, but if the auger chamber has a smaller diameter, the cleaning tool must be sized appropriately.)
- WD-40
- Rifle cleaning rod (.22) with slotted end, with small fabric cleaning patches
- Aqueous solution of Triton X-100(1%), minimum size 1 liter
- Isopropyl alcohol solution of Triton X-100 (1%), minimum size 1 liter
- Two containers per crew for the solutions tall enough to hold the auger and extractor
- Clean dry cloth for drying
- Recloseable plastic bags (sandwich bag size) for holding core samples
- Cooler with ice or cooling block

References

Gordon, M. 2008. Field methods for the detection of *Bridgeoporus nobilissimus* DNA in trees, stumps, and snags. Final report submitted to USDI Bureau of Land Management, Oregon State Office. Contract number HAP084563. On file with: Interagency Special Status/Sensitive Species Program, USDI Bureau of Land Management, Oregon State Office, Portland, Oregon; USDA Region 6 Forest Service, Regional Office, Portland, Oregon. <http://www.fs.fed.us/r6/sfpnw/issssp/documents/inventories/inv-rpt-fu-brno-field-methods-for-detection-2008-12-09.pdf>

Decay classes:

					
code >	1	2	3	4	5
Bark	Tight, Intact	50% loose or Missing	75% Missing	75% Missing	75% + Missing
Heartwood decay	Minor	None to Advanced	Incipient to Advanced	Incipient to Advanced	Advanced to Crumbly
Sapwood decay	None to Incipient	None to Incipient	None to 25%	25%+	50%+ Advanced
Limbs	Mostly present	Small Limbs Missing	Few remain	Few remain	Absent
Top breakage	May be present	May be present	Approx. 1/3	approx. 1/3-1/2	approx.1/2+
Bole form	Intact	Intact	Mostly Intact	Losing form, soft	Form mostly lost
Time since death	1-5 years	> 5 years	-	-	-

Limiting Distances

Limiting Distance to Face of tree

1. Measure DBH (nearest tenth).
2. Measure % slope to face of tree at DBH.
3. "Combined factor" x DBH = limiting distance
4. If measured distance from plot center to face of tree is less than limiting distance, tree is in.

% of Slope	Slope Correction Factor	Plot Radius Factor * Slope Correction Factor "Combined Factor"					
		5 BAF	10 BAF	15 BAF	20 BAF	30 BAF	40 BAF
1	1	3.847	2.708	2.203	1.902	1.546	1.333
2	1.0002	3.848	2.709	2.203	1.902	1.546	1.333
3	1.00045	3.849	2.709	2.204	1.903	1.547	1.334
4	1.0008	3.85	2.71	2.205	1.904	1.547	1.334
5	1.00125	3.852	2.711	2.206	1.904	1.548	1.335
6	1.0018	3.854	2.713	2.207	1.905	1.549	1.335
7	1.00245	3.856	2.715	2.208	1.907	1.55	1.336
8	1.00319	3.859	2.717	2.21	1.908	1.551	1.337
9	1.00404	3.863	2.719	2.212	1.91	1.552	1.338
10	1.00499	3.866	2.722	2.214	1.911	1.554	1.34
11	1.00603	3.87	2.724	2.216	1.912	1.555	1.341
12	1.00717	3.875	2.727	2.219	1.916	1.557	1.343
13	1.00841	3.879	2.731	2.222	1.918	1.559	1.344
14	1.00975	3.884	2.734	2.224	1.921	1.567	1.346
15	1.01119	3.89	2.738	2.228	1.923	1.563	1.348
16	1.01272	3.896	2.742	2.231	1.926	1.566	1.35
17	1.01435	3.902	2.747	2.235	1.921	1.568	1.352
18	1.01607	3.909	2.752	2.238	1.933	1.571	1.354
19	1.01789	3.916	2.756	2.245	1.936	1.574	1.357
20	1.0198	3.923	2.762	2.245	1.94	1.577	1.359
21	1.02181	3.931	2.767	2.251	1.943	1.58	1.362
22	1.02391	3.939	2.773	2.256	1.947	1.583	1.365
23	1.02611	3.947	2.779	2.261	1.952	1.586	1.368
24	1.0284	3.956	2.785	2.266	1.956	1.59	1.371
25	1.03078	3.965	2.791	2.271	1.967	1.594	1.374
26	1.03325	3.975	2.798	2.276	1.965	1.597	1.377
27	1.03581	3.985	2.805	2.282	1.97	1.601	1.381
28	1.03846	3.995	2.812	2.288	1.975	1.605	1.384
29	1.0412	4.005	2.82	2.294	1.98	1.61	1.388
30	1.04403	4.016	2.827	2.3	1.986	1.614	1.392

From plot center to center of tree:

Table 13. Common Basal Area Factors and Angle Sizes Used in Point Sampling

Basal area factor (ft ² /ac) ^a	Basal area factor (m ² /ha)	Angle size (min)	Angle size (dipters)	Ratio (tree diameter to plot radius)	Plot radius factor (ft) ^b	Plot radius factor (m) ^c
5	1.15	73.66	2.14	1/46.7	3.889	0.467
10	2.30	104.18	3.03	1/33.0	2.750	0.330
15	3.45	127.59	3.71	1/26.9	2.245	0.269
20	4.60	147.34	4.29	1/23.3	1.944	0.233
25	5.75	164.73	4.79	1/20.9	1.739	0.209
30	6.90	180.46	5.25	1/19.0	1.588	0.190
35	8.05	194.92	5.67	1/17.6	1.470	0.176
40	9.20	208.38	6.07	1/16.5	1.375	0.165
50	11.50	232.99	6.79	1/14.8	1.230	0.148
60	13.80	255.23	7.44	1/13.5	1.123	0.135

^aft²/ac converted to m²/ha by multiplying by the approximate conversion factor 0.23.

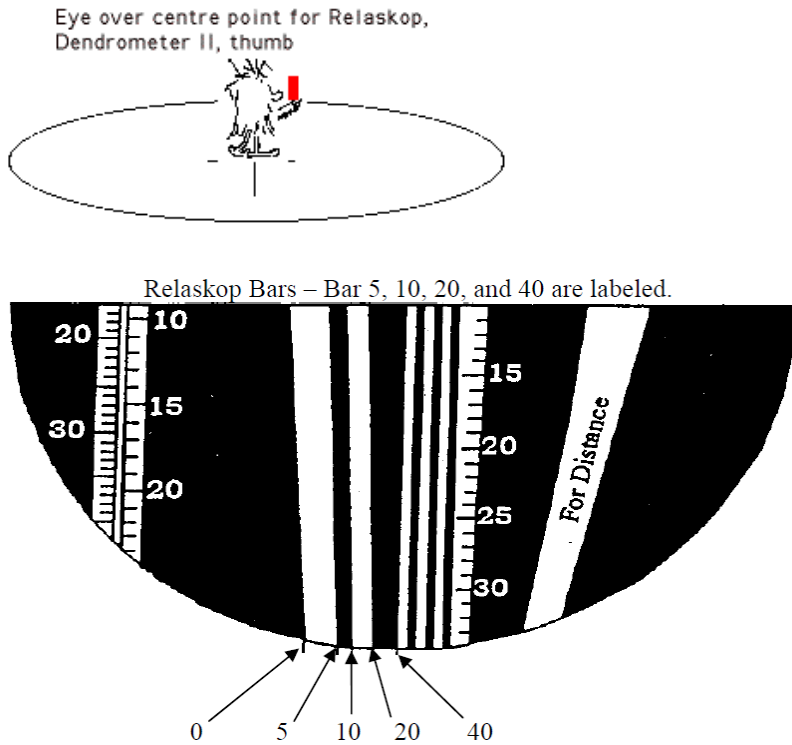
^bLimiting distance in feet equals PRF times dbh in inches.

^cLimiting distance in meters equals PRF times dbh in centimeters.

Variable plot sampling

Spiegel relascope

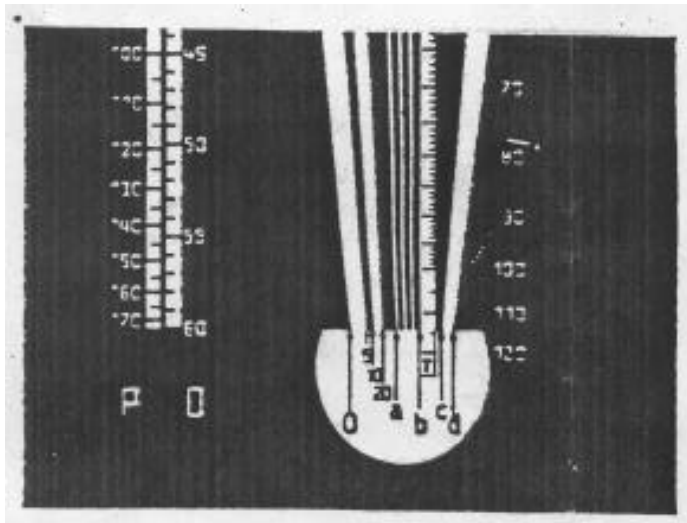
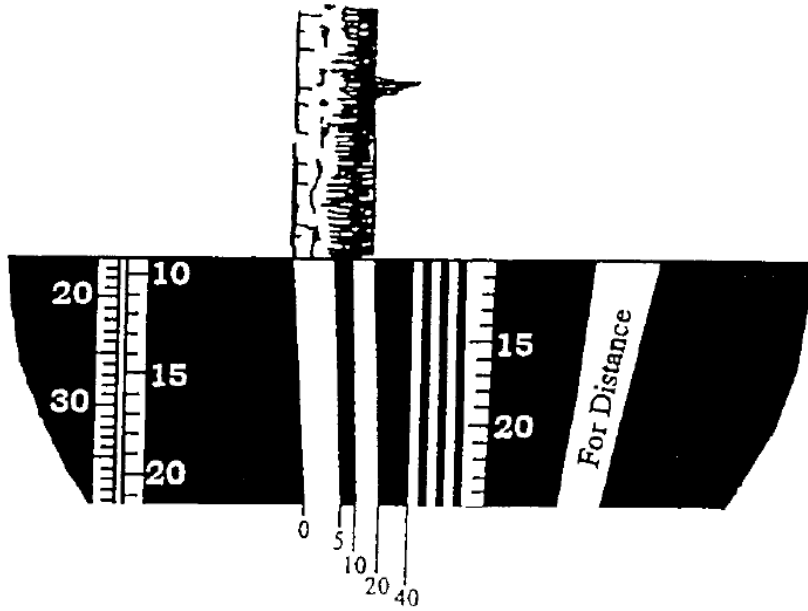
If a Spiegel relascope is used, the person's eye is held over the plot center. Trees are counted "in", when they are wider than the predetermined number of stripes inside the viewing field. The number of stripes used depends on the BAF used for the plot. Use the same procedure as the wedge prism to determine if a tree is "in" or "out."



Looking through the Relaskop at DBH, the tree must be **larger** than the width of the bars for any given

BAF to be considered **In**.

This tree is **In** using a 5 or 10 BAF, **borderline** using a 20 BAF, and **Out** using a 40 BAF.



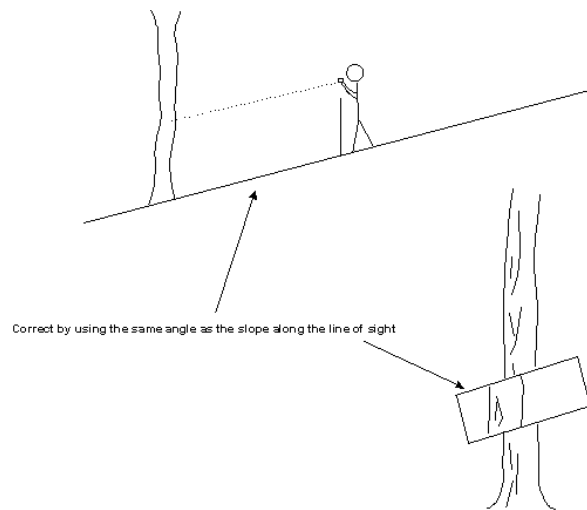
Wedge prism

If a wedge prism is used, the prism is held over the plot center and the person moves around the prism. When using a prism, the distance from the eye to the prism is not important as long as the tree can be seen clearly.

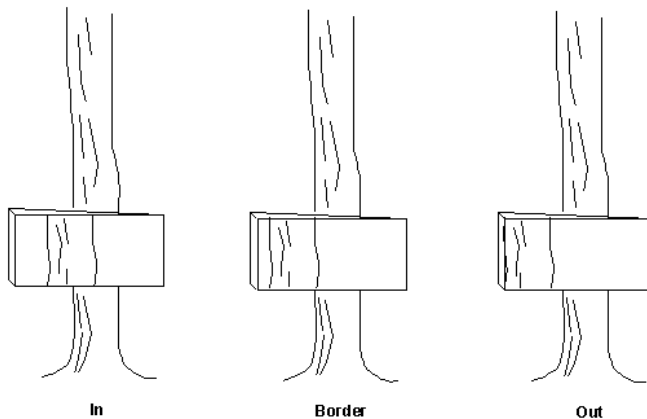
Wedge over centre point.
Keep it centred and walk in a circle



Where more than 10 percent slope is involved between the prism and the tree, correct for slope. The way to slope correct with a prism is by turning the prism at an angle parallel to the slope along which you are looking and sight to DBH. Slope correction are only necessary up and down slopes.



A tree is determined “in” if their shifted image in the prism still overlaps with the rest of the trunk. It is “out” if the image does not overlap and it is a borderline tree if the image lines up with the edge of the tree.



To determine if a borderline tree is “in” or “out,” measure the diameter breast height (DBH) of the tree and the distance from the plot center to the center of the tree. Compare the distance against the limiting distance table for the appropriate BAF and dbh.

References:

<http://sres-associated.anu.edu.au/mensuration/acs.htm>
 Forestry Handbook by Karl F. Wenger, Society of American Foresters
http://forest.mtu.edu/classes/fw2051/docs/point_intro.doc
 USDA Forest Service Timber Cruising Handbook
<http://oak.snr.missouri.edu/nr3110/topics/vps/vps.html>
http://forest.wisc.edu/facstaff/Radeloff/No11_variable_area_plots_I.pdf
<http://www.fs.fed.us/r6/nr/fp/Measurements/Cruising/basiccruise0808.pdf>
http://www.fs.fed.us/im/directives/field/r4/fsh/2409.12/2409.12_90.doc