

## **BACKGROUND AND OBJECTIVES**

This survey is voluntary and designed to gather information on the distribution of the pathogen *Phytophthora ramorum* (Pr), cause of the disease known as Sudden Oak Death (SOD). The survey strategy reflects the current understanding of the biology and ecology of Pr, known hosts and potential hosts based on laboratory testing or taxonomic similarity, and likely pathways for its introduction. The best available science was used to determine appropriate risk factors and level of risk. As knowledge of Pr host susceptibility, biology, and epidemiology changes or improves, the factors used in the risk assessment will also change.

The purpose of the survey is to detect the presence of Pr. It is neither a population survey nor an attempt to express the amount of area affected by this pathogen. Additional delimiting and evaluation surveys will be done around newly detected infestations to estimate affected area and associated impacts.

Pr is a quarantine pest and may have been inadvertently introduced to all states outside the regulated areas in CA and OR on infected nursery stock in 2003-04. Special care to disinfect boots or vehicles after sampling a location is required when surveying around a suspect positive location. Care should be taken to disinfect tools used in scraping oak bark for revealing stem canker symptoms before sampling another to avoid inadvertent transmission of pathogens from tree to tree (e.g. oak wilt). Cutting tools such as knives or pruning shears require disinfestation before moving from one transect to the next.

## **RISK-BASED SAMPLING POLYGON DEVELOPMENT**

Forest Health Monitoring has produced a National map identifying sampling polygons based on risk (Figure 1).

The following factors were used to assign risk and develop the sampling polygons:

1. Presence of known Pr host species, host genera, and closely related genera.  
Overstory: Percent basal area of *Quercus* species in the red oak and live oak groups (eastern United States) or presence of known hosts (western United States).  
Understory: Only the evergreen understory hosts were considered in this analysis. Presence of actual evergreen understory hosts (western United States) or number of evergreen host species (eastern United States).
2. Locations of nurseries receiving Pr host stock.  
Current analysis based on rhododendron stock.
3. Length of yearly mesic/moist weather period.

- One, two, or three months with five inches of rain or three inches of rain and two days dense fog when temperature is between 60 to 80 degrees F.
- Area outside limiting temperature extremes currently associated with Pr.  
Extreme temperature limits are defined as a minimum of one month with winter maximum temperature of less than 32 degrees F or one month with summer maximum temperature greater than 90 degrees F.

The smaller the polygon, the higher the perceived SOD risk.

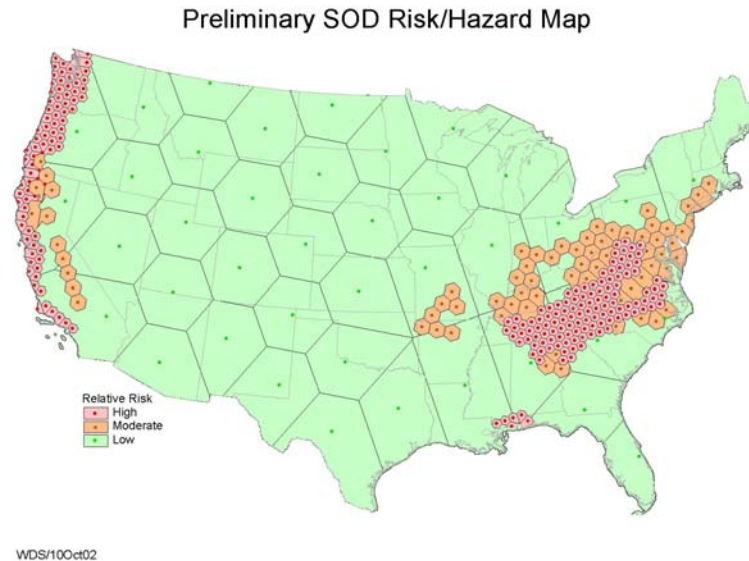


Fig. 1. Preliminary SOD Risk Map

Risk as projected in Figure 1 can be modified by known or suspected importation of Pr host nursery stock or other plant materials from infested areas elsewhere in the world. Where suitable host type vegetation occurs in proximity to such nurseries, risk is elevated.

### **FOREST SURVEY REQUIREMENTS**

Sampling should be concentrated in high risk forested environments; however, a minimum sampling of low risk forest environments is encouraged if time and funding permit.

### **Polygon Sampling**

Thirty locations is the target number of sample sites for each state participating in the survey. More sampling may be possible or less may be required, depending on the number of nurseries or other areas receiving potentially infested plant materials, budget constraints, etc. Detailed maps of risk polygons, road networks, and other landmarks are available by state in the form of ARCVIEW compatible files from Steve Oak, USDA Forest Service, Southern Region- FHP, Asheville, NC.

A sampling decision flow diagram is presented in Figure 1A to aid surveyors in prioritizing various nursery and forest situations for survey.

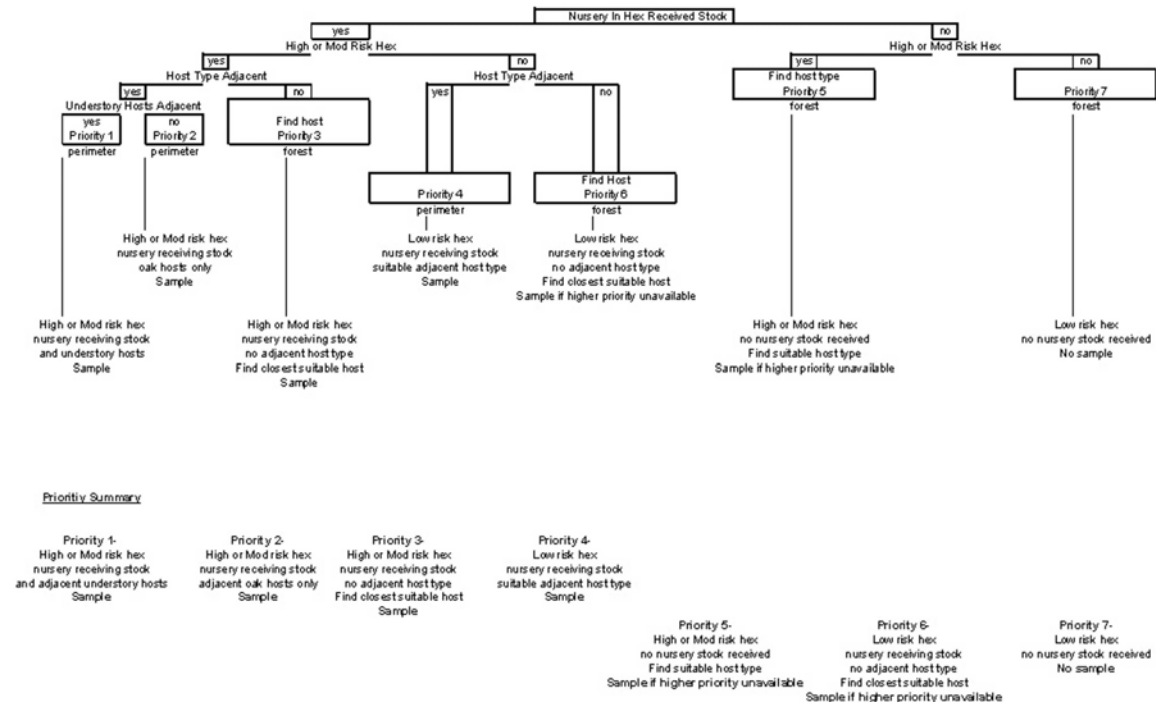


Figure 1A. Survey priority decision flow diagram for Pr.

Highest priority should be given to sampling in high risk polygons in forested areas adjacent to the nurseries that have received plant materials potentially infected with Pr. In states where only a small number of nurseries are available for sampling, the remaining locations will be distributed among forested areas adjacent to other nurseries that are not known to have received such material, the general forest area, and lower risk classes. Sample sites should be geographically dispersed whenever logistically possible. Close cooperation with State Plant Regulatory Official (SPRO) in your state will be essential to determine the nurseries to be surveyed and other nurseries not receiving potentially infected materials but willing to be included in the forest survey, as well as in obtaining owner permissions for private land access.

**Important:** Some sampling of the general forest not associated with nurseries should be done in each state, if time and funding permit.

### Forested areas around nurseries

#### Sampling Scheme

Information concerning the name and location of nurseries is obtained through the SPRO in each state. The perimeter of the nursery with suitable host type adjacent will be identified and surveyed with four 100 meter transects distributed so as to sample all available aspects while ensuring that the microclimate most conducive to disease

development (cool and moist) is surveyed. Thus, the plot location is selected based on the presence of attributes suitable for disease with regard to host type and environment (i.e., purposive rather than random). Suitable host type is defined any forest type with a significant component of the following plant genera: *Acer*, *Aesculus*, *Castanea*, *Fagus*, *Hamamelis*, *Kalmia*, *Lonicera*, *Quercus*, *Rhododendron*, *Vaccinium*, and *Viburnum*. Where available, give preference to forest types with a significant oak component. To be sampled as a nursery perimeter setting, this combination of overstory/understory must occur within 0.25 miles of the nursery production field. Sample the entire nursery perimeter if the total length is 400 meters or less.

On arrival at a nursery site, scout the nursery production areas for suitable host type and aspects to determine the particulars of available transect sites. This is not an area-based survey. Transect width is a function of the detection limit, i.e. how far you can see, which can vary according to the density of vegetation.

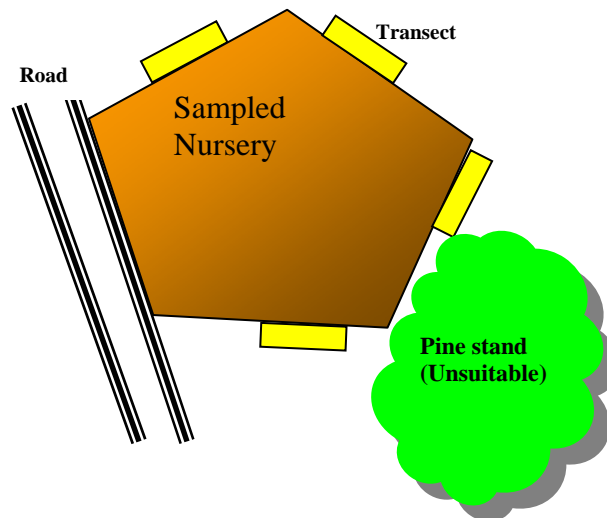
Target species/tissue types are:

Leaves:

*Acer*, *Aesculus*, *Hamamelis*, *Kalmia*, *Lonicera*, *Rhododendron*, *Vaccinium*, *Viburnum*

Bleeding Stem Canker:

*Quercus*, *Castanea*, *Fagus*



On the field form (attached), record observer name, date, nursery perimeter setting, hex number, state FIPS code, county name, location name, and location number. Number locations consecutively. For each transect, record starting point witness tree information, starting and ending GPS coordinates (as well as any turning points with interim distances that may be necessary along the nursery perimeter), overstory composition, and understory species. Scout for hosts and symptoms while traversing the transect.

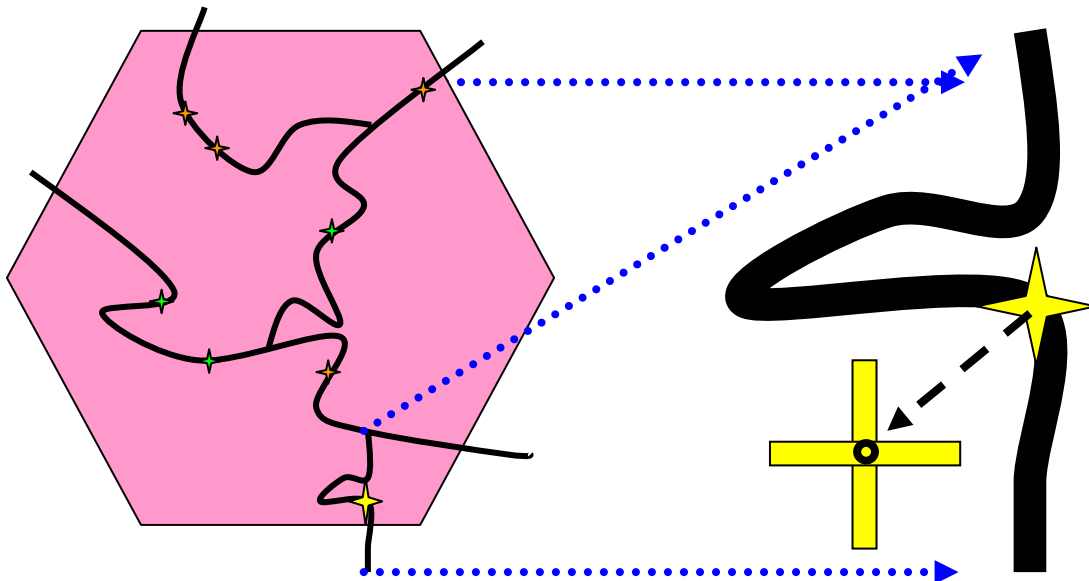
Collect any suspect **bleeding stem cankers**, distinguishing from seeps associated with stem boring insects, oak wilt, or other stem cankers. Carefully slice away outer bark to reveal symptomatic inner bark beneath with a drawknife or hand axe. Chip off a 5 X 5 cm section of bark that includes the active canker margin. Wrap sample in plastic food wrap (e.g. Saran Wrap) and place in a plastic bag with a pre-printed label (provided) inside and out. **Double bag all samples.** Record state, hex number, transect number, location number and name abbreviation, host species code, and tissue type code on both labels and the field data sheet. **Double bag host species separately if more than one is collected on a transect.** Codes are found on the reverse side of the field data sheet. **Collect replicate samples on half of all transects at each location to be processed at the PCR lab at Mississippi State University as a quality control measure.** While in the field, keep samples cool on sealed coolant bags. Disinfect any tools used to scrape suspected stem cankers with Lysol spray after each use and before moving on to a new candidate canker.

If **foliar hosts** are available, collect a maximum of 50 symptomatic leaves or shoots on each transect, keeping samples of each host species separate if more than 1 is available. Disperse sample collection along length of transect if symptoms are present throughout. Otherwise collect as available. Collect no more than 5 leaves from individual plants/clumps. When the length of the transect is traversed, examine all collected samples and select the 10 best symptomatic leaves of each host. Priority should be given to *Phytophthora* leaf and twig lesions if present, but do not limit collection to those. Recognize that most lesions will not resemble “typical” lesions caused by *Phytophthora* species. Place samples of each host in plastic bags with pre-printed labels (provided) inside and out. **Double bag all samples.** Record state, hex number, transect number, location number and name abbreviation, host type code, and tissue type code on both labels and the field data sheet. **Double bag host species separately if more than one is collected on a transect.** Codes are found on the reverse side of the field data sheet. A clean, dry paper towel should be added to bags containing leaf samples to absorb excess moisture. Disinfect cutting tools used in leaf and twig collection before beginning sampling of a new transect. **Collect replicate samples on half of all transects at each location to be processed at the PCR lab at Mississippi State University as a quality control measure.**

Keep samples cool on sealed coolant bags while in the field and mail with any stem canker samples as soon as possible to the PCR diagnostic laboratory via overnight mail on sealed coolant bags in a special mailer (provided). Include a “Chain of Custody” form (provided) in a sealed plastic bag showing the sample ID’s included in that mailing. It is permissible to collect samples on Friday so long as they are mailed overnight (i.e. for Monday morning arrival; maximum 72 hours after field collection). If Monday morning arrival cannot be guaranteed, then samples must be frozen until they can be mailed overnight the following week.

### **General forest areas not adjacent to nurseries**

Although the sample location in forested areas not associated with nurseries is random, the plot location is purposive, i.e. the plot has attributes suitable for the disease with regard to host type. Once at a potential sample location, field crews will assess suitability of host type and the size of the area available for sampling. Travel to the next potential sample location if it's unsuitable. If it's suitable, walk off the road into the stand at least 100 meters to a plot center. On the field data sheet (attached), record observer name, date, general forest area setting, hex number, state FIPS code, county name, location name, and location number. Number locations consecutively. Record GPS coordinates and witness tree data at the starting point, and install four 100 meter transects on cardinal azimuths from this plot center. The location of transects on the ground may be modified from cardinal directions to capitalize on optimal available host type and microclimate most conducive to disease. Use a compass to initiate the travel routes and the GPS unit to assist in navigation. This is not an area-based survey. Transect width is a function of the detection limit, i.e. how far you can see, which can vary according to the density of vegetation.



**High-risk hex with road/forest type intersection.**

✧ Point where road network intersects forest cover .

✦ Suitable host type.

✧ Unsuitable host type.



**Road segment w/ sample point selected by field crew.**

Traverse from road point 100 meters minimum into suitable forest type to install plot center. Four sample transects are 100 meters long by 10 meters wide @ cardinal azimuths from plot center.

Suitable host type selected for field sampling.

Fig. 2 Road/polygon intersection showing sample points location.

Traverse the entire length of the transect scouting for hosts and symptoms. Delay collection of leaf samples of foliar hosts, but collect any suspect bleeding stem cankers immediately; distinguish from seeps associated with stem boring insects, oak wilt, or other stem cankers. Chip off a 5 X 5 cm section of bark that includes the active canker margin. Wrap sample in plastic food wrap (e.g. Saran Wrap) and place in a plastic bag with a pre-printed label inside and out. **Double bag all samples.** Record state, hex number, transect number, location number and name abbreviation, host species code, and tissue type code on both labels and on the field data sheet. **Double bag host species separately if more than one is collected on a transect.** Codes are found on the reverse side of the field data sheet. **Collect replicate samples on half of all transects at each location to be processed at the PCR lab at Mississippi State University as a quality control measure.** While in the field, keep samples cool on sealed coolant bags. Disinfect any tools used to scrape suspected oak stem cankers with Lysol spray after each use and before moving on to a new candidate canker.

After scouting the length of the transect for understory hosts and symptoms, collect available symptomatic foliage samples of understory host materials on the return travel route to the plot center. Collect a maximum of 50 symptomatic leaves and 10 shoots on each transect from foliar hosts, keeping samples of different species separate if more than one is available. Disperse sample collection along length of transect if symptoms are present throughout. Otherwise collect as available. Collect no more than 5 leaves from individual plants/clumps. When the length of the transect is traversed, examine all collected samples and select the 10 best symptomatic leaves of each host available. Priority should be given to *Phytophthora* leaf lesions if present, but do not limit collection to those. Recognize that most lesions will not resemble typical lesions caused by *Phytophthora* species. Place the 10-leaf composite samples of each host in plastic bags with pre-printed labels (provided) inside and out. **Double bag all samples.** Record state, hex number, transect number, location number and name abbreviation, host species code, and tissue type code on both labels and the field data sheet. **Double bag host species separately if more than one is collected on a transect.** Codes are found on the reverse side of the field data sheet. **Collect replicate samples on half of all transects at each location to be processed at the PCR lab at Mississippi State University as a quality control measure.** A clean, dry paper towel should be inserted in bags with leaf samples to absorb excess moisture. Disinfect cutting tools used in leaf and twig collection before beginning sampling of a new transect.

Keep samples cool on sealed coolant bags while in the field and mail with any stem canker samples as soon as possible to the PCR diagnostic laboratory via overnight mail on sealed coolant bags in a special mailer (provided). Include a "Chain of Custody" form (provided) in a sealed plastic bag showing the sample ID's included in that mailing. It is permissible to collect samples on a Friday so long as they are mailed overnight (i.e. for Monday morning arrival; maximum 72 hours after field collection) If Monday morning

arrival cannot be guaranteed, then samples must be frozen until they can be mailed overnight the following week.

### **HANDLING PLANT SAMPLES**

Collect samples as described above. **Disinfect** any tools used to scrape suspected oak stem cankers after each use and before moving on to a new candidate canker with Lysol spray. Disinfect other cutting tools before beginning sampling a new transect. Special care to disinfect boots or vehicles after sampling a location is required when surveying around a suspect positive location. .

**Keep different host plant species collected on the same transect in separate double bags from each other. Do not transport plant parts or bark samples unless double bagged, labeled inside and out and sealed, along with an accompanying “Chain of Custody” form.** A clean dry paper towel should be inserted in bag with leaf samples to absorb excess moisture. Bark samples should be wrapped in plastic wrap, double bagged and labeled inside and out with preprinted labels (provided). All samples should be protected from direct sunlight and kept in a cooler on a sealed coolant bag or in a refrigerator until shipped. Mail samples to the appropriate diagnostic lab via overnight mail. Contact information for PCR diagnostic labs can be obtained from your Regional FHM Coordinator.

Northeastern Region:

Jim Steinman (610) 557-4158 [jsteinman@fs.fed.us](mailto:jsteinman@fs.fed.us)

Northcentral Region:

Manfred Mielke (651) 649-5267 [mmielke@fs.fed.us](mailto:mmielke@fs.fed.us)

Southern Region:

Jim Brown (404) 347-2471 [jdbrown@fs.fed.us](mailto:jdbrown@fs.fed.us)

Interior West Region:

Ralph Thier (801) 625-5820 [rthier@fs.fed.us](mailto:rthier@fs.fed.us)

West Coast Region:

Alison Nelson (503) 808-2976 [asnelson@fs.fed.us](mailto:asnelson@fs.fed.us)

**If samples cannot be mailed so as to be received by the diagnostic lab within 72 hours of collection, they should be frozen and mailed on sealed coolant bags as soon as practical.** A completed chain of custody form should be sealed in a plastic bag and placed inside the container used to ship samples to the PCR diagnostic labs.

### **Laboratory Protocols**

**Diagnostic laboratories should report results to the Regional FHM Coordinators no less frequently than every 2 weeks. Regional FHM Coordinators should then**



**forward those results to the SPRO, SPHD, and state forest health cooperator in the state where the samples originated; the APHIS National Nursery Program Manager (Dan Williams); the National SOD Forest Survey Technical Coordinator (Steve Oak); and the National FHM Program Leader (Borys Tkacz).**

False positive PCR results occasionally occur. In the case of an initial positive PCR result, the processing laboratory should repeat the reaction using surplus DNA from the initial extraction and/or from DNA extracted a second time from the stored sample. If the positive result persists after these tests, then the laboratory should contact the QA/QC laboratory (Dr. Susan Diehl, Mississippi State University) to determine if the sample has a replicate. If positive PCR results are accepted after consultation and retesting, Inform the SPRO and SPHD in the state where the sample originated as well as the Regional FHM Coordinator. **Confirmation of *Phytophthora ramorum* finds can only be completed by a diagnostic laboratory approved by APHIS.** Therefore, repeat sampling of the transect yielding the positive sample will be necessary for additional plant samples to be used for culturing. Inform Dr. Laurene Levy (USDA-APHIS Beltsville, MD) of the pending shipment and send new samples.

**Dr. Laurene Levy  
USDA APHIS PPQ CPHST  
Plant Germplasm Quarantine and Biotech Laboratory  
Bldg 580, BARC-East  
Beltsville, MD 20705**

**(301) 504-7157  
laurene.e.levy@aphis.usda.gov**

**The replicate samples collected on half of the transects at each sampling location should be sent to:**

**Dr. Susan V. Diehl  
Associate Professor  
Department of Forest Products  
Mississippi State University  
662-325-3101 (office)  
662-325-8126 (FAX)  
email: [sdiehl@cfr.msstate.edu](mailto:sdiehl@cfr.msstate.edu)**

### **Data Compilation and Analysis**

**IMPORTANT!!** Field crews will send a summary spreadsheet form every 2 weeks to their **Regional FHM Coordinator**. The data required in the summary report include: Date of report, Cumulative number of nursery perimeters surveyed, Cumulative number of nursery perimeter samples submitted for diagnostics, Cumulative number of forest locations surveyed, and the Cumulative number of forest location samples submitted for

diagnostics. **Regional FHM Coordinators will add negative and positive PCR results and in turn forward summary reports to the SPRO, SPHD, and state forest health cooperator in the state where the samples originated; the APHIS National Nursery Program Manager (Dan Williams); the National SOD Forest Survey Technical Coordinator (Steve Oak); and the National FHM Program Leader (Borys Tkacz) on the same schedule.**

**At the end of the survey, each state should transfer data to the ACCESS Database and forward the files to the Regional FHM Coordinator, who will then forward copies of all datasheets for entry into the NAPIS database to Jim Pheasant, NAPIS Database Manager Purdue University (765) 494-9853; and to the National SOD Forest Survey Technical Coordinator (Steve Oak).**

**Sudden Oak Death Forest Survey vE 04/22/04**

**Observer Name(s)** \_\_\_\_\_

**Date** \_\_\_\_\_

**(Circle One) Nursery Perimeter    General Forest Area**

Hex Number	State FIPS	County

Location Name	Location #

Transect 1 (N)	Latitude	Longitude	Distance
Starting Coordinates			
Turning Point 1			
Turning Point 2			
Turning Point 3			
Ending Coordinates			
<b>Host Type: (check all that apply)</b>			
Predominantly Oak Forest	<b>Starting Point Witness</b>		
Mixed Oak Forest (some oak)	Species		
Other Overstory	DBH		
Mt. Laurel Understory	Azimuth		
Rhododendron Understory	Distance		
Other Host Understory			

Transect 1 Sample Bag ID	Sample
Hex No./Loc. No./Transect No./Loc. Name	Spp./Tissue Type

Transect 2 (E)	Latitude	Longitude	Distance
Starting Coordinates			
Turning Point 1			
Turning Point 2			
Turning Point 3			
Ending Coordinates			
<b>Host Type: (check all that apply)</b>			
Predominantly Oak Forest	<b>Starting Point Witness</b>		
Mixed Forest (some oak)	Species		
Other Overstory	DBH		
Mt. Laurel Understory	Azimuth		
Rhododendron Understory	Distance		
Other Host Understory			

Transect 2 Sample Bag ID	Sample Type

Transect 3 (S)	Latitude	Longitude	Distance
Starting Coordinates			
Turning Point 1			
Turning Point 2			
Turning Point 3			
Ending Coordinates			
<b>Host Type: (check all that apply)</b>			
Predominantly Oak Forest	<b>Starting Point Witness</b>		
Mixed Forest (some oak)	Species		
Other Overstory	DBH		
Mt. Laurel Understory	Azimuth		
Rhododendron Understory	Distance		
Other Host Understory			

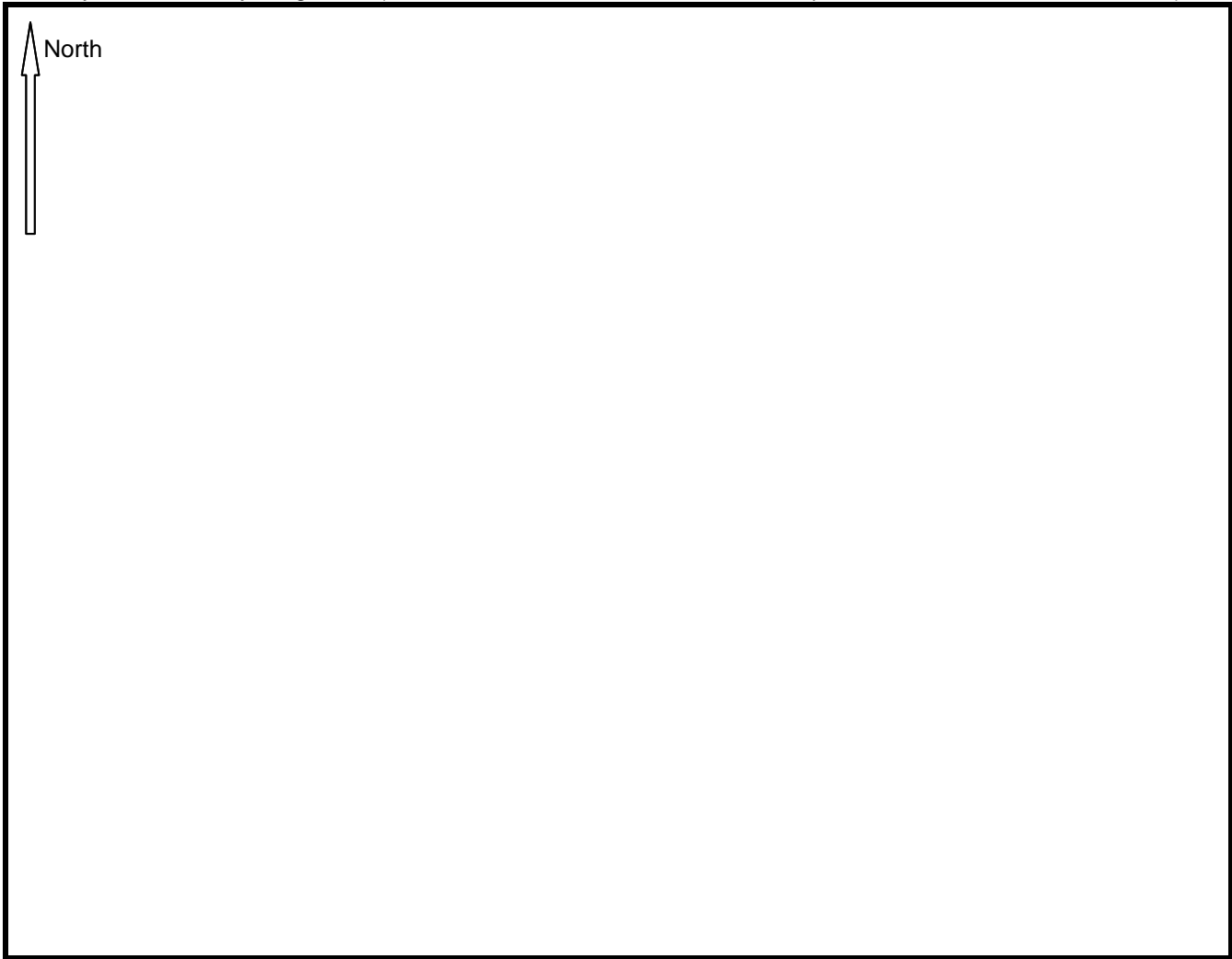
Transect 3 Sample Bag ID	Sample Type

Transect 4 (W)	Latitude	Longitude	Distance
Starting Coordinates			
Turning Point 1			
Turning Point 2			
Turning Point 3			
Ending Coordinates			
<b>Host Type: (check all that apply)</b>			
Predominantly Oak Forest	<b>Starting Point Witness</b>		
Mixed Forest (some oak)	Species		
Other Overstory	DBH		
Mt. Laurel Understory	Azimuth		
Rhododendron Understory	Distance		
Other Host Understory			

Transect 4 Sample Bag ID	Sample Type

*Sketch nursery boundaries or forest features and relative transect locations on reverse side of form*

Nursery/Forest Vicinity Diagram (show relative locations of transects and production fields or forest features)



**Sample Coding- xxxxxxx/x/x/xxxxx/xxx/xxx**

Hex number- 7 characters  
 Location number- 1 character  
 Transect number- 1 character  
 Location name- 5 characters  
 Understory/Oak Species Code- 4 or 3 characters  
 Tissue Type code - 2 or 3 characters

**Tissue Type Code**

LF = Leaf  
 TW= Twig  
 BAR = Bark

**Understory Code**

2105	honeysuckle
2092	mt. laurel
2135	rhododendron
2205	vaccinium
2210	viburnum
2071	witch hazel

**Oak Species Codes**

802	white	823	burr	833	no. red
804	sw. white	824	blackjack	834	shumard
806	scarlet	825	sw.chestnut	835	post
812	so. red	826	chinkapin	837	black
813	cherrybark	827	water	838	live
819	turkey	828	nuttall	899	unk. oak
820	laurel	831	willow		
822	overcup	832	chestnut		

**Other Tree Species Codes**

310	maple
330	buckeye
421	chestnut
531	beech