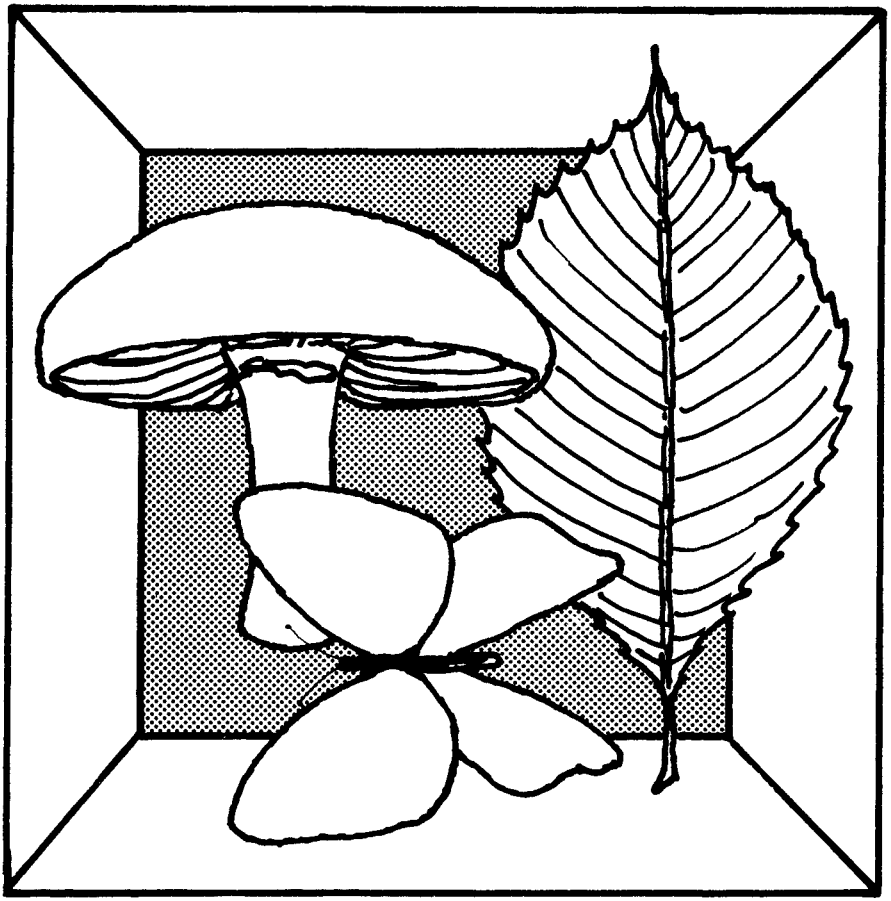
HOW TO COLLECT AND PREPARE FOREST INSECT & DISEASE ORGANISMS AND PLANT SPECIMENS FOR IDENTIFICATION



U. S. Department of Agriculture – Forest Service – Southeastern Area – State & Private Forestry

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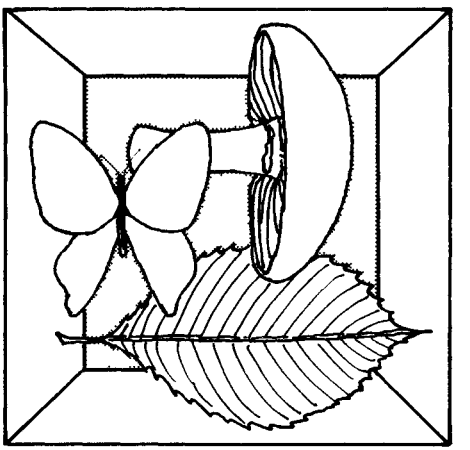
General Report SA-GR 13

HOW TO COLLECT AND PREPARE FOREST INSECTS, DISEASE ORGANISMS AND PLANT SPECIMENS FOR IDENTIFICATION

By
William H. Hoffard, Entomologist,
Robert L. Anderson and William H. Sites, Pathologists*

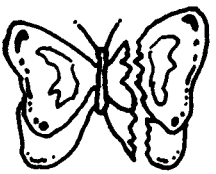
USDA FOREST SERVICE
Southeastern Area, State and Private Forestry
1720 Peachtree Road, N.W.
Atlanta, Ga. 30309

*The authors are stationed at the Asheville, N.C., Field Office.



CONTENTS

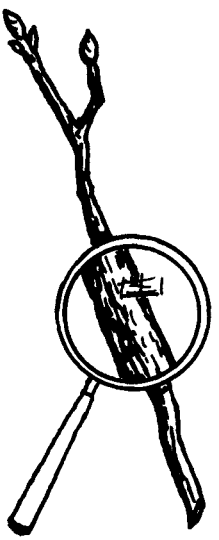
INTRODUCTION



Forest insect and disease specimens often must be sent to distant locations for identification. Almost invariably, poorly prepared or badly packaged specimens arrive damaged – often to the point that identification is impossible. You can avoid the disappointment of a notice asking you for another specimen (which you may not be able to get) by using this guide to collect, prepare and ship your specimens.

Specimen preservation, packaging and shipping is simple, but requires care. Techniques vary among different kinds of insects and disease samples. The recommendations in this guide apply to most types of specimens, or to techniques which can be generally applied to them all. For a more thorough discussion, consult the list of suggested readings.

PLANT SPECIMEN COLLECTION



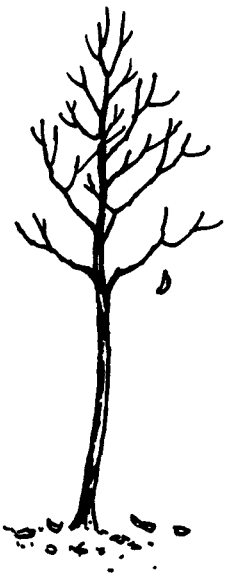
If a tree appears to be unhealthy, examine it and its environment closely. Examine other trees of the same species in the vicinity as well as those of other species. Determine, if possible, whether other similarly affected trees occur in groups or are scattered more or less randomly through the stand.

Next, check the immediate surroundings of the affected trees. Look for disturbances of soil and other vegetation and evidence of underground systems such as utility lines. Observe a wide area and adjacent farmland, if any, for evidence of herbicides or other pesticide use. These environmental factors are especially important in shade tree disease diagnosis. Be as specific as possible about the presence of streets, buildings, and cultural practices such as pruning.

Look at the symptoms. Has the tree suddenly turned brown or has it defoliated gradually while remaining green? Has the tree begun to decline from the top down? Has the bark loosened? Did the tree wilt while still green? Are the leaves curled? All of these and any other similar "expressions" by the tree are called **symptoms** and are the first consideration in

diagnosis and possible specimen selection. While studying them, think back to conditions over the past year. Were there extremes in temperature, droughts of any consequence or other factors detrimental to plant growth and development?

While examining the tree for symptoms, look also for *signs*. **Signs** are physical evidence of causes, i.e., insects and disease organisms. Fruiting bodies, such as conks and mushrooms, are the signs produced by fungi. They may be found growing on the host tree itself, on the ground around it or on nearby stumps and logs. Examples of insect signs include frass and cast larval skins, and the insects themselves. Carefully collect and prepare suspicious specimens for shipment, using the suggestions given later in this booklet. Not all fungi and insects in the vicinity of the unhealthy tree may be the cause of the problem. Try to establish some relationship between the affected tree, its unhealthy or dead neighbors and the specimen(s) you collect.



Smaller trees are easier to examine than large ones and may permit a more thorough field diagnosis. No matter how large or small, however, examine the entire tree, if possible. When present, foliage can be an invaluable aid. However, it may only be symptomatic of a problem centered elsewhere. For example, wilted leaves or top dieback do not necessarily indicate the presence of foliar insects or diseases, but may instead result from the effects of a stem or root pest. Check the host for loose or dead bark, for cankers on limbs and stems, insects or insect parts, and for root and root collar damage. However, do not submit bark by itself unless cankers, fruiting bodies, or possibly characteristic insect damage are present on it. Many organisms inhabit the bark and thus isolation from this material produces much contamination.

If possible, insects to be collected should be killed in the field. Soft bodied specimens such as aphids and beetle larvae may simply be dropped in alcohol. Put moths and most hard bodied insects in a killing jar. The best killing jars are wide-mouthed, with about ¾ inch (19 mm) of hardened plaster of paris in the jar bottom. A few drops of ethyl acetate (nail polish remover) are added to the plaster of paris where they are quickly absorbed. Some tissue paper added to the killing jar will help prevent moths from damaging their wings against the jar sides and bottom.

INSECT SPECIMEN PREPARATION

Adult Insects

Relaxing

If insects are dead long before pinning, rigormortis develops, often resulting in a body distortion.[1] In such cases, use a "relaxing chamber" to make them flexible. Relaxing chambers are very simple and inexpensive to construct. One type is simply an airtight covered dish which contains moist paper towels or damp sand (figure 1). A small amount of fungus inhibitor (such as Lysol®) should be added.

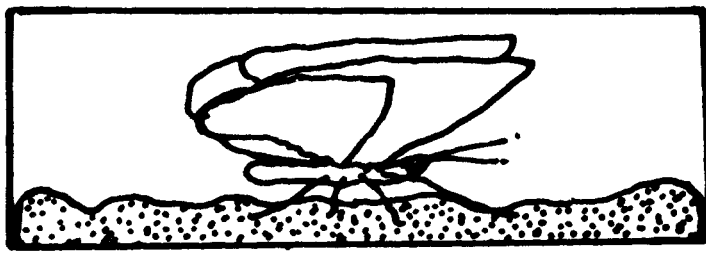


Figure 1

When insects are removed after the relaxation period (36-48 hours), they should be pliable. At this time, their appendages can be more attractively or practically positioned - usually with temporarily placed insect pins. Rigormortis again sets in, but this time the insects are "frozen" so that their important identification characters are more discernible.

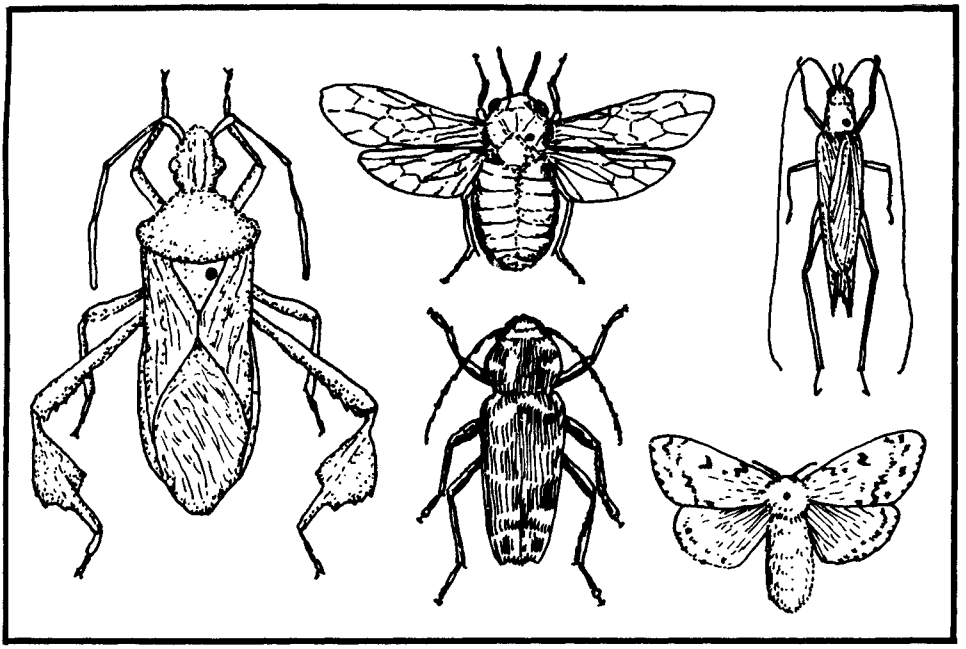
Pinning

In most cases, adult insects should be pinned. Use pins specifically designated as "insect pins." Pin placement varies with different kinds of insects. Large, hard-bodied insects, such as most long-horned, wood boring beetles, are pinned directly through the body. Pin positioning varies slightly with the classification of the insects (beetles, flies, moths, true bugs, etc.). Figure 2 shows proper pinning areas for more common types of larger insects.

Small insects, such as bark beetles, cannot accommodate large pins. Therefore, they must be placed on "points," which in turn are pinned. Points are triangular, tiny pieces of rigid

[1]Shipment of live insects is beyond the scope of this publication (see section, "Miscellaneous Information")

Figure 2 . – Black dots represent proper pinning areas for insects of various classifications. (The specimens are not drawn to scale.)



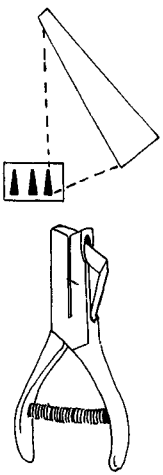


Figure 3

paper. They can be made with scissors, or more easily with a point maker. Point makers resemble hole punches and quickly punch out uniform-sized points (figure 3). To position bark beetle-sized insects on a point, the small end of the point is bent down and a tiny drop of glue is applied to the deflection. Then the insect is attached at its right side (figure 4).

Do not use too much glue as it may obscure the middle and opposite side of the insect. Ordinary glue is acceptable but a clear acetate cellulose cement (such as ambroid) is preferable because it does not become too brittle. Ambroid is available at variety stores.

Insects that are still smaller, such as some wasps and flies, are mounted on unbent points. Minute insects (such as mosquito-sized parasitic wasps and tiny moths) are mounted on tiny pins which are inserted in cork, which is then pinned with a regular insect pin.

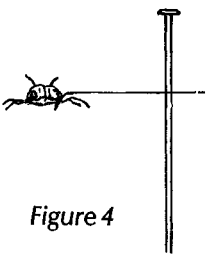


Figure 4

Mount insects on pins or points a height of 1 inch (2.5 cm) above the base of the mounting. This height can easily be determined with a pinning block (figure 5). Pinning blocks can be homemade or purchased from biological supply houses. Specimen height is simply equal to the maximum

depth reached by the pin in the deepest of three holes. The function of holes 2 and 3 will be discussed under "labeling."

Because of their structure, moths require special treatment. While still pliable, or after relaxation, pin the moth directly through the middle of the thorax and position it on a spreading board (figure 6). Then pin paper strips over each set of wings parallel to the torso. Gently spread the wings with a needle so that they are fully extended. If a spreading board is

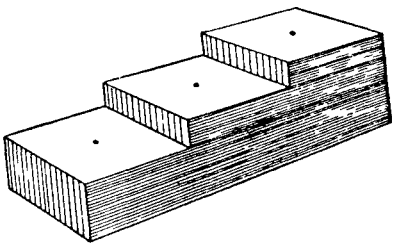


Figure 5

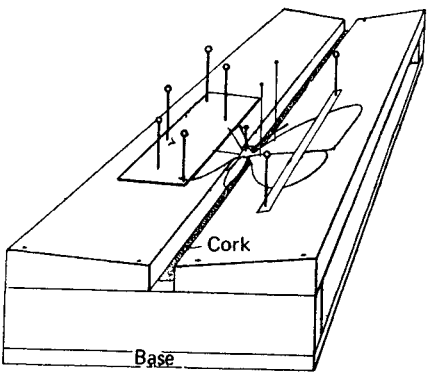


Figure 6

not available, this same basic procedure can be used on a broad, flat surface, but position the insect upside down to avoid leg damage. When the abdomen feels firm to the touch, the specimen has sufficiently dried and can be labeled.

As mentioned earlier, not all adult insects should be pinned. Soft-bodied specimens (such as aphids and scales) must be preserved in alcohol.

Immature Insect Preparation

Most immature insects should be preserved in alcohol. Make every effort to use *ethyl* alcohol. If ethyl is unavailable, rubbing alcohol may be used for temporary preservation. Liquid preservation is obviously practical for such insects as moth and sawfly larvae. Here too, forethought will help ensure proper preservation. Several liquid formulae are used to preserve specimens. A common, simple and functional mixture is 95 parts of 70-80 percent ethyl alcohol with five parts glycerine. Glycerine is easily obtainable from drugstores. In-

sects so preserved are usually kept in vials with solid rubber stoppers.

If possible, kill the insect by dipping it in boiling water before preserving it in a vial. This treatment helps ensure better color retention. Immediately after death, put the insect in a full vial of the alcohol-glycerine mixture. A specimen label should accompany the insect (labeling will be discussed in detail, later). With an insect pin placed against the interior of the vial, force the rubber stopper deep into the vial as the pin is simultaneously withdrawn. This procedure pulls surplus air out of the vial as a stream of tiny bubbles, thus helping to ensure an airtight fit (figure 7).

Because the body contents of a large specimen may dilute the preservative, it is best to replace the original alcohol with fresh alcohol after a day or two.

As mentioned earlier, not all immature insects should be preserved in alcohol. Hard-bodied nymphs, for example (e.g., conebugs), should be pinned in the same manner as adults.

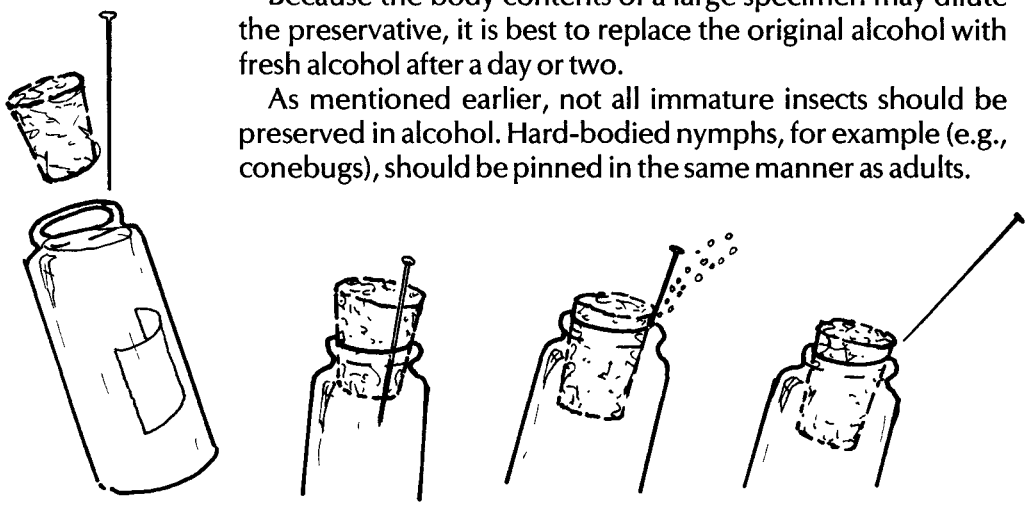


Figure 7

Insect Labeling

Proper labeling is an integral part of specimen submission. The insect identifier uses the collection date and location shown on the label to help narrow down or confirm identification. Figure 8 is an enlarged example of a typical label. Line 1 shows the collection place. Lines 2 and 3 are for the collection date and collector's name, respectively.

ASHEVILLE, NC.
AUG 20, 1979
J.P KROUS-COLL.

Figure 8

If the insect is pinned through its body, the location label is pinned through the center and pushed to level 2 (or ¾ inch) above the pinning block. With specimens on points, move the pin toward the beginning of the label to provide better balance of the insect and label.

Level 3 of the pinning block is for the specimen identification label. It is not necessarily used by the submitters unless they request confirmation of an identification. Additional information such as host data or specimen number may also be placed on label 3.

With labeling, too, proper care ensures functional, long-lasting data. Print the letters in block style. Labels should be very small, but neat. They may be printed in either India ink or pencil (preferably India ink). Because the lettering is so small, many workers prefer to use rapidograph pens. Fountain pens are impractical and fiber points are unacceptable. Labels for alcohol-preserved specimens are simply included loosely in the vial. The alcohol will not affect quality ink or lead penciling.

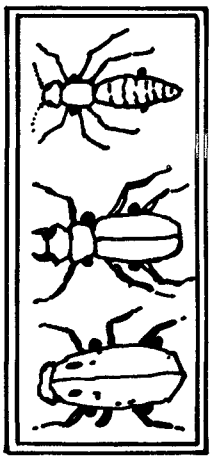


Figure 9

Insect Packaging

Poor specimen packaging is an endless source of frustration for those assigned responsibility for insect identification. Package your insect specimens to withstand rough handling during shipment. Protection against rough handling should be made first within the specimen area itself. With pinned specimens, the pinning base should be tight, thick styrofoam or a similar material. Fit the base tightly in a box bottom. Also glue it to the box with a strong nonvolatile adhesive which would not corrode the styrofoam or box. Press the pin deeply into the styrofoam, but not to the point where it protrudes or begins to protrude through the box bottom. If specimens are large, use stabilizing pins which prevent them from turning in transit (figure 9).

Line the box edges with cotton which has been securely glued or pinned. Its purpose is to catch any insect appendages which may become dislodged in transit, thus preventing further damage. Fit the lid firmly on the box. Ideally, it too is fitted with a styrofoam inner top which fits securely over the knobs of the pins.

The exterior should show instructions on how to remove the lid (e.g., "lift straight up" or "hinged here" followed by arrows). Under no circumstances send pinned and bottled specimens in the same container. Wrap each vial separately in cloth or paper towels.

FUNGAL FRUITING BODY COLLECTION AND PRESERVATION

A disease specialist can best identify a fruiting body correctly, if you follow certain steps. When submitting the fleshy, mushroom type, collect fresh, mature and immature fruiting bodies, typical of those observed. Collect at least six representative samples. While fresh, make a spore-print (figure 10) from each sample. First, cut the mushroom cap from the stalk. Place the cap with gills or pores down on a clean piece of paper that is half white and half black and then cover it with an inverted glass jar (figure 11). Some experimentation may be necessary to determine when the print is definitive. Set up several caps on the paper sheets and remove them after varying periods. Allow the caps to air dry, then, fold papers so the sport-print does not rub off. Do not dry over heat.

Figure 10

Fleshy fungi shrivel and shrink upon drying – so they will not resemble their fresh condition. Before they dry, make note of the basic shape, size and color of all parts (e.g., cap, stalk, gills) and include other information about the specimen and host.

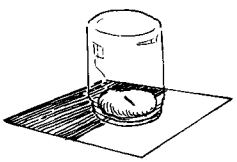


Figure 11

Perennial conks are usually woody and require no particular handling for preservation. Fleshy fungi are quite another matter and must be given special care. Rapid drying until brittle in a drying chamber is necessary immediately after collection. Quick drying prevents other fungi, bacteria, and insects from destroying the specimen. The chamber can be made by placing a screen over two 100 watt bulbs. The screen and bulbs should be in a fire resistant box with an open top (figure 12).

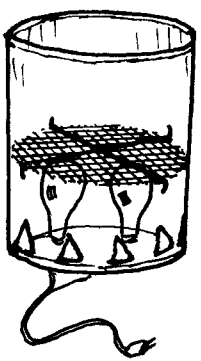


Figure 12

Put dried fungal material in a paper bag for storage and shipment. Plastic bags will not permit residual moisture to escape. This moisture may permit the growth of destructive bacteria and saprophytic fungi. The following flow chart summarizes the procedure for preparation and shipment of fresh fruiting bodies:

Mushroom → Make spore-print → Dry → Paper Bag → Box → Ship

COLLECTION AND PRESERVATION OF PLANT MATERIAL AND SOIL

When possible, collect whole plants along with some of the soil from around the roots. If the plant is too large for collection, examine and collect samples from different parts. In addition, collect samples from other plants showing different stages of the problem and from at least one that is healthy.

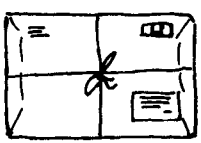
When collecting a branch or stem sample, take a wedge or section that includes both dead and apparently healthy tissue. This advancing margin frequently contains the living pathogen.

Keep all plant materials moist and cool. Place host material in a plastic bag with moist paper toweling and put it in a cool place. Vehicles parked in the sun in the summer can become hot enough to kill fungal pathogens. As a result, they cannot be isolated from the host.

Put a sample of the soil in a plastic bag, but separate from the associated plant specimen. Keep the soil cool before shipment.

SHIPPING OF INSECT AND DISEASE SPECIMENS

Ship all material – including insects, fungi, plant material and soil – in a sturdy carton or mailing tube. Cartons are preferable for insect specimens. Pad the interior container with 2 to 3 inches (5 to 8 cm) of packing material surrounded by another box. Make sure it is protected from moisture. Include in the specimen box a description of the contents, host and host environment, your address and that of the recipient. Forest Service form 3400-1 (figure 13) is one convenient checklist that can be used. Seal the container with cord or fiber tape. Place the words "CONTENTS: PLANT MATERIAL (or DEAD INSECTS) OF NO COMMERCIAL VALUE" on the package. Keep boxes with perishable specimens in a cool place until delivery to the carrier. If living plants or pests are among the contents, obtain a permit from the USDA's Animal and Plant Health Inspection Service (APHIS) and attach it to the package.



US DEPARTMENT OF AGRICUTURE
Forest Service

Form Approved
OMB No 040 R 2844

DETECTION REPORT
Forest Insect and Disease Damage
(Reference FSM 3420)

PART A INSTRUCTIONS *Th s report is authorized by PL 95 313 Voluntary responses to the report greatly assist the Forest Service in the detection and prevention of potentially destructive insects and diseases PRINT Shaded areas to be completed by receiving Agency Futher instructions on inside cover*

1 TYPE OF LANDOWNERSHIP (X Appropriate Box)
☐ Federal ☐ State ☐ Private

2 UNIT (Indicate specific National Forest National Park etc)

3 SUBUNIT (Indicate District or other appropriate subunit)

4 LOCATION OF PROPERTY (If known give drainage name prominent landmark mileage from known location)

State _____ County _____

Township | Range | Section | ¼ Section

5 NAME AND BUSINESS ADDRESS (Include Zip Code)

6 PHONE NUMBER (Include Area Code)

7 DATE

8 ACTION REQUESTED (X Appropriate Box)
☐ None ☐ Identification ☐ Field Evaluation

9 SAMPLE ENCLOSED	10 MAP ENCLOSED	11 ACRES DAMAGED	12 TREES DAMAGED	13 DISTRIBUTION OF DAMAGE
☐YES ☐NO	☐YES ☐NO			☐ Scattered ☐ Grouped

14 SPECIES AFFECTED (Indicate species in descending order of damage severity)

15 TREE SIZE (X Appropriate Box)	16 PARTS(S) OF TREES DAMAGED (X Appropriate Box)	17 STAND TYPE (X Appro Box)
☐ 20 Seedling	☐ 30 Root ☐ 35 New Foliage	☐ 40 Nursery
☐ 21 Sapling	☐ 31 Bole ☐ 36 Old Foliage	☐ 41 Plantation
☐ 22 Pole	☐ 32 Branch ☐ 37 Bud	☐ 42 Natural
☐ 23 Sawtimber	☐ 33 Twig ☐ 38 Cone/Seed	☐ 43 Shelterbelt
☐ 24 Overmature	☐ 34 Leader ☐ 39 Wood	☐ 44 Ornamental
		☐ 45 Seed Orchards

18 CASUAL AGENTS OR ASSOCIATED DISTURBANCE (X Appropriate Box)

Insects	Disease	Mechanical	Other
☐ 50 Bark Beetle	☐ 60 Root	☐ 70 Blowdown	☐ 75 Hail	☐ 80 Herbicide
☐ 51 Defoliator	☐ 61 Foliage	☐ 71 Thinning	☐ 76 Snow	☐ 81 Air Pollution
☐ 52 Sapsucking	☐ 62 Decays	☐ 72 Logging	☐ 77 Frost	☐ 82 Salt
☐ 53 Borer	☐ 63 Canker	☐ 73 Drought	☐ 78 Wind	☐ 83 Animal
☐ 54 Other	☐ 64 Other	☐ 74 Flood	☐ 79 Fire	☐ 84 Bird

Indicate casual agent(s) if known _____

19 REMARKS (Enter any pertinent information to indicate severity of damage or symptoms to help identify casual agent Use Table I on instruction sheet above as an aid in describing symptoms)

PART B TO BE COMPLETED BY RECEIVING AGENCY

1 IDENTIFICATION OF CASUAL AGENT (Reply by Entomologist/Pathologist)

2 INFORMATION REPORTED (X Appropriate Box)
☐ Reared/Cultured ☐ Sample Damaged Resubmit ☐ Sent out for identification

3 REMARKS

4 REPORTED BY (Signature)	5 DATE	6 REPORT NUMBER

FS 3400 1(3/79)

Figure 13 – Forest Service Form 3400-1.

If you have perishable specimens or a large number of preserved insects, contact the receiver to arrange for a convenient shipping date. Then, ship by the fastest means possible and early in the week to ensure the specimens are not held by the carrier over the weekend.

MISCELLANEOUS INFORMATION

Shipment of Live Pests

Packaging and shipment of live insects is beyond the scope of this publication. Refer to *Entomological Techniques: How to Work with Insects,* by Alvah Peterson, for ideas. The shipment of live insect pests is subject to regulations of the Animal and Plant Health Inspection Service.[2]

Acquiring Supplies

There are numerous biological supply houses throughout the United States which supply equipment needed for preserving and shipping insects. *An Introduction to the Study of Insects,* by Borror, DeLong and Triplehorn contains a list. Local high school biology departments, extension agents, and colleges also can suggest suppliers. Ordinarily, no special equipment is necessary for disease specimen preparation.

Where to Send Specimens

Forest Service employees should submit their specimens to entomologists or pathologists with Forest Insect and Disease Management (State and Private Forestry). Homeowners and state agency foresters should check with their State Forester or county extension agent regarding specimen submission.

[2]Information and permits are available from USDA Animal and Plant Inspection Service, Plant Protection and Quarantine, Hyattsville, Md. 20782

SUGGESTED READING

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