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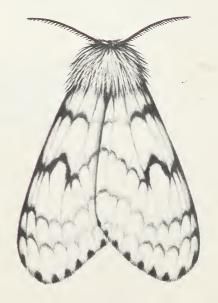
Combined Forest Pest Research and Development Program

Agriculture Information Bulletin No. 418

7 85

Gypsy Moth Handbook

Judging Vigor of Deciduous Hardwoods



### Contents

In 1974, the U.S. Department of Agriculture initiated the
Combined Forest Pest Research
and Development Program, an
interagency effort that
concentrated on the Douglas-fir
tussock moth in the West, on the
southern pine beetle in the
South, and on the gypsy moth in
the Northeast. The work
reported in this publication was
funded in whole or in part by the
program. This manual is one in a
series on the gynsy moth.

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USDA, National Agricultural Library NAL Bldg 10301 Baltimore Blvd Beltsville, MD 20705-2351 Judging Vigor of Deciduous Hardwoods by Philip M. Wargo<sup>1</sup>

#### Introduction

Tree vigor can be defined as an individual tree's physiological condition that results from its physiological performance within a particular environment. Every tree is unique and responds to the environment in its own way. Thus, for a given set of environmental conditions, a range of physiological performances—and therefore a range of physiological conditions or tree vigors—exists.

Within a species, vigor categories of crown position (dominant, intermediate, or suppressed) or crown condition (good, fair, or poor) indicate a tree's past relative growth and general vigor, but they indicate little about a tree's current vigor and vulnerability to the effects of stress. When stressed by defoliation, for example, trees in all the above vigor categories may die, or crown condition may deteriorate (Campbell and Valentine 1972), indicating that within these general vigor categories there are gradations of tree health.

Tree vigor is a major factor in determining disease and insect resistance of trees that have been stressed and in predicting the ability of trees to tolerate stresses such as defoliation, drought, and air pollution. Methods that measure tree vigor precisely are needed to predict accurately the effects of stress on trees and add to our ability to make better pest management decisions.

A good indicator of tree vigor should reflect the tree's present physiological condition, normally not be subject to rapid fluctuation, be sensitive to stress, and be easy to measure. Two such techniques have these properties: The measurement of root starch content and stem electrical resistance.

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Starch is the major form of reserve carbohydrate in most deciduous trees and reflects the photosynthetic activity (or capacity) of the tree—an indicator of the well-being of a tree (Kramer and Kozlowski 1960), High starch content in a tree indicates that growing conditions were favorable and photosynthesis was sufficient to maintain all living tissues plus store high amounts of food. High starch is then equated with high or good vigor. Low or depleted starch indicates insufficient photosynthesis and is equated with low or poor vigor.

In deciduous hardwood trees, starch is stored heavily in the roots and is a good indicator of the physiological performance of trees (Wargo et al. 1972). Starch content of the roots is three to five times higher than in the stem (Jones and Bradlee 1933, Murneek 1942, Wenger 1953, Wargo 1971). When quantative changes in starch content occur in the tree, they are of larger magnitude in the

roots than in stems and are easier to detect. Root starch is also more stable than that in the stem. The soil buffers the roots from wide temperature fluctuations that can cause rapid changes in the amount of starch maintained in the tissue (Ewart et al. 1954) Starch content is affected by stress: Defoliation (Staley 1965, Parker and Houston 1971, Wargo 1972, Wargo et al. 1972), drought (Parker 1970), and air pollution (Miller et al. 1968) can cause reductions in the starch content. Research on sugar maple showed that defoliation had an immediate effect on starch content and that at least one full growing season was required for trees to

recover after one defoliation (Wargo

and frequency of defoliation (Wargo

stress, starch content is also easy to

1972, Wargo et al. 1972). Starch

content also reflected the severity

et al. 1972). In addition to being a

reliable and sensitive indicator of

measure (Wargo 1975).

## How to Measure Starch Content (Histochemical Technique)

Root Sampling

Starch content can be determined colorimetrically after extracting the starch from the wood (Siminovitch et al. 1953. Hassid and Neufeld 1964), and histochemically by staining root tissues with potassium iodide solution (Lugol's Stain, Johansen 1940), (Wargo 1975), The chemical extraction techniques have the advantage of being more quantitative than the histochemical method, but they take more time and equipment and must be done in a laboratory. The histochemical technique, although less precise, is simpler and faster and can be done in the field. It can even be used to compare roots of different diameters that may differ in their amounts of ray tissue, which is the major starch storage tissue in deciduous trees (Wargo 1976). This eliminates the need to sample from roots of the same diameter or the same distance from the stem base. It is this technique that seems to be best for use by land managers and is described here

Root tissue from the trees to be analyzed can be collected any time between autumn after leaf fall and spring before bud activity when the ground is not frozen. (Roots can be sampled when the ground is frozen, but it is more difficult to dig.) During this period, starch content is normally highest and stable in the roots. A single root sample indicates the starch status of the tree (Wargo 1976).

A small section of root wood is removed with a suitable chisel and hammer about 30 cm distal to the soil line on the root (figs. 1 and 2). The bark can be removed first to





Figure 1.—Soil-root line on buttress root.
Figure 2.—Buttress root excavated and ready for sample removal.

facilitate root wood removal. The piece of wood should be as small as practical to facilitate wound closure. A section of root wood 1.5 cm wide and 1.5 cm long cut to form a wedge has proven to be a suitable size (fig. 3, *A* and *B*). If tissues are dry and sections tend to shatter, remove the bark and make shallow cuts at the top, bottom, and sides of the wedge. Add water to the well created by removing the bark and let it soak into the woody tissue, then chisel out the wedge.

Root samples can be analyzed immediately or they can be stored in an insulated container with ice and analyzed later. If the root samples are not going to be processed within 48 hours of collection, they should be frozen until analyzed.





Figure 3.—A, Removing sample of root tissue with hammer and wood chisel; B, wedge of root tissue used for starch determination.

#### **Root Sampling Procedure**

Subsequent work on root sampling has shown that root samples can be taken with a standard increment borer. The wound created in the roots with a borer is smaller and closes faster than a wound created with a chisel or an arch punch. Use of the borer also eliminates tissue shattering that sometimes occurs when roots are sampled when dry.

Cores should be taken from root tissue below the soil line. This procedure avoids problems of starch to sugar conversions that can occur in above ground tissues when diurnal temperature fluctuates above and below freezing in autumn and early spring — the usual time for root sampling. For efficient sugar bush management, root sampling and starch rating should be done in the autumn so that the decision whether to tap can be made well in advance of the sugaring season.

A 2- to 3-cm-long (approx. 1 inch) core yields sufficient root wood for making sections to stain for starch content. Orient the core with the growth rings facing directly upward, and cut the sections for staining across the rings. If the whole core

is not cut, cut sections from the youngest growth rings (closest to the bark), but cut at least 1 cm (0.5 inch) of core. Although it is best to use a microtome for cutting root sections for staining, sections can also be cut freehand with a straight razor, razorblade, or scalpel. The important factor is that sections from every tree should be of similar thickness so that ratings are consistent. Sections of different thicknesses may be rated differently even though they have similar starch contents.

The publication "Estimating starch content in roots of deciduous trees -A visual technique," by Philip M. Wargo (1975), USDA Forest Service Research Paper NE-313, can be used as a guide for rating starch content. Storage of starch is a dynamic process in plants, and starch content. even in healthy trees, may fluctuate from year to year. Therefore, trees rated for starch content in any given vear should be rated against trees that are considered healthy and unstressed for that year. This procedure will indicate how severely a tree, forest stand, or sugar bush was affected by a stress, such as defoliation by thrips.

#### **Preparing and Staining Root Sections**

Roots are prepared for staining by cutting thin cross sections of the root piece with a suitable slicer. Sections can be cut on a number of types of microtomes available from most biological supply houses. The two major types for cutting sections from woody material are the tabletop microtome on which sections

are cut with a hand-held knife and the sliding microtome on which sections are cut mechanically (fig. 4, *A* and *B*). The table top model is smaller and preferred for field use (fig. 5).

The root piece should be thawed if frozen, washed free of soil, debarked if necessary, and trimmed





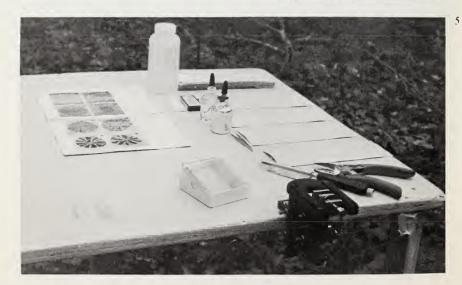


Figure 4.—A, Sliding microtome; B, tabletop hand microtome. Figure 5.—Tabletop microtome set up for field use.

4 B

to fit the tissue holder on the microtome. Slice cross sections. from the root piece until complete sections can be cut. Then cut three sections 75 to 100 microns thick and place them on a glass slide. The key to cutting sections is how uniform in thickness the sections are, not necessarily how thin they are. Slicing is easier if the root piece is kept moist. Immediately flood the sections with I<sub>2</sub>KI staining solution—1.5 g potassium iodide (KI) plus 0.3 g crystalline iodine (I) dissolved in 100 ml distilled water (fig. 6). The staining solution should be kept out of sunlight and

refrigerated between daily uses and made fresh every 30 days. Blot up excess stain, reflood the sections with fresh stain, and let the solution remain for 5 minutes. Blot or rinse away excess stain and rinse until clear with clean water. Examine the sections for uniformity of staining pattern. If no two sections are alike, cut additional sections. If two are alike, the starch content can be rated



Figure 6.—Slides showing stained and unstained cross sections of root wood.

#### **Rating Sections for Starch Content**

Remove all but one section from the slide. Flood the section with water or glycerine and place a cover slip or another slide over the section to prevent the section from drying before it is rated. Hold the slide 1 cm or more above a white background or an opaque transmitted light source, and examine the section with the naked eve, a hand lens, or a dissecting binocular microscope. The stained section can then be rated for starch content as high, medium, low, or depleted (fig. 7). Starch is stored primarily in the ray tissue; in species such as oak, in which ravs are very

prominent, emphasis should be placed on the amount of starch in them. The stained sections can be compared to relative standards such as those shown in the Forest Service visual technique publication (Wargo 1975). Another reference standard could be stained sections from similar trees that were known to be unstressed.

Stained sections should be rated immediately for starch content for best results. If not rated within 48 hours after staining, they should be restained.

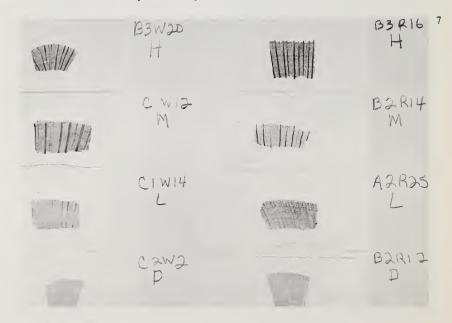


Figure 7.—Series of cross sections from roots of red (right) and white (left) oaks showing the four major categories of starch content: High (H), medium (M), low (L), and depleted (D).

## Risk Rating Trees According to Starch Content

The relationship of starch content to vigor provides a basis for risk rating trees; the risk refers to the potential for decline and mortality if the tree is defoliated during the next growing season. Trees high in starch are considered high vigor, low-risk trees; trees with a medium amount of starch are considered moderate-vigor, moderate-risk trees; and trees with low or depleted starch are considered low-vigor, high-risk trees.

This method was developed to determine the vigor of individual trees. By combining the vigor ratings of trees from sampling areas that adequately represent the species and area, or stand of interest, the vigor of a stand can be indicated, as was done in the following field test.

## Field Test of Histochemical Starch Technique

A field test of the histochemical starch technique was conducted in 1975 in a gypsy moth infested area in Bald Eagle State Forest in central Pennsylvania. Nine plots, each with 25 red oaks (including Ouercus rubra L., O. velutina Lamarck, and O. coccinea Muenchh) and 25 white oaks (including O. alba L., and O. prinus L.) were established on the fringe of a gypsy moth infested area. The area had been reported as having had little defoliation in 1974. but heavy defoliation was anticipated in 1975. The field trial served two purposes: to test the practicality of the histochemical technique and to determine the relationship of risk rating (based on starch content), defoliation, and tree mortality.

Using the techniques previously described, a two-person crew easily sampled over 50 trees per day.

#### **Predicting the Effects of Defoliation**

The root samples were analyzed later in a field laboratory. A two-person crew sliced, stained, and rated about 60 root samples per day. Inexperienced crews were trained in one day; using the histochemical technique manual (Wargo 1975) as a guide, they rated the root sections as accurately as an experienced crew.

The analysis clearly revealed that the available information on previous defoliation for the area was inaccurate. Starch content of the trees indicated that the stands had been stressed and that the white oaks were low in vigor. In the white oak group, 161 of the 225 trees had low or depleted starch reserves. which indicated that the trees had been heavily defoliated for 1 and possibly 2 years prior to 1975. Yet the information from aerial sketch maps indicated only light defoliation for the area in 1974. It is likely that in this mixed stand of red and white oaks, the white oaks were defoliated preferentially during population buildup of the gypsy moth when overall defoliation was light. The lack of heavy defoliation of the red oaks masked the defoliation incurred by the white oaks.

Predictions of mortality cannot be based solely on starch determinations. Preliminary analysis of results from the field test indicated that starch content percent of defoliation, crown condition, percent of slope, and percent of trees that were white oak accounted for much of the variation in tree mortality. In addition, previous stand disturbance and abundance of secondary organisms, such as Armillariella mellea (Vahl ex Fr.) Karst., a root-attacking fungus, and Agrilus bilineatus (Webb), the twolined chestnut borer. which are the dominate causes of mortality after defoliation, must be considered (Dunbar and Stephens 1975, Kegg 1973, Nichols 1968, Houston and Valentine 1977, and Wargo 1977).

While precise and accurate estimates of mortality cannot be based on starch content alone, an estimate of starch content can be used as an indicator of the effects of previous environmental conditions and of a tree's or stand's potential for damage should additional defoliation occur. This information, plus other vigor criteria such as crown position, crown condition, and stand age, can be readily incorporated into evaluations of the impact of defoliation and pestmanagement decisions.

#### Electrical Resistance

Another technique that is still under development but that shows promise as a method for judging tree vigor utilizes the resistance of stem tissue to electrical current. Electrophysiological relationships in trees have been studied for a long time (Tattar and Blanchard 1976). However, the recent development of a small, portable, resistance meter that delivers a pulsed electric current has stimulated considerable recent investigation of the relationship of electrical properties to vigor (Shigo and Shigo 1974). The meter, named the Shigometer, was initially developed to detect discoloration and decay in living trees and wood in service such as utility poles (Skutt et al. 1972, Shigo and Shigo 1974).

The meter has been used with modified probes to measure electrical resistance (ER) of the inner bark-outer wood area of stems to show a relationship between ER and vigor. In general, studies have indicated that vigorous trees have lower ER and that ER increases when trees are stressed. ER has been related to categories of crown class and condition and frequency and severity of defoliation (Wargo and Skutt 1975), to wound closure in sugar maple (Wargo 1977), to sprout vigor in red maple and wound closure in hybrid poplar (Shortle et al. 1977), to fertilization of white birch (Shigo et al. 1975), and to symptoms of vascular wilt diseases (Carter and Blanchard 1976, Malia and Tattar 1975). No relationship was found between declining crown conditions of urban

maples and ER, or between nursery trees stressed by drought, root pruning, or soil compaction and ER. In nonurban maples, however, the vigorous trees had the lower ER readings (Newbanks and Tattar 1977).

The major difficulty in interpreting ER measurements is that numerous tree properties, such as sapwood wood thickness, bark thickness, radial growth, ion concentration, and stage of growth seem to influence the electrical measurements and cause considerable variation in electrical resistance. Research is continuing on the use of electrical techniques to measure tree health, but currently no technique can be considered operational.

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