FOREST WATCH

STUDY GUIDE

Introduction
&
Protocols

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The Forest Watch Program

STUDENTS AND SCIENTISTS WORKING TOGETHER DETERMINING THE HEALTH OF NEW ENGLAND FORESTS

In New England, we are surrounded by forests. When people think of New England, they think of some aspect of our forests: brilliant fall colors, maple sugaring, skiing through forested glades, distant mountain vistas, all covered by forests. The forests of New England are so much a part of our surroundings that we may be tempted to take our forests for granted. At a time in our planet's history when human-generated greenhouse gases are increasing in our atmosphere at an alarming rate, we must begin to think of our forest as an important part of controlling the atmospheric level of one of these greenhouse gases: carbon dioxide (CO_2).

Forests actively remove CO_2 from the atmosphere through the process of photosynthesis, and store this CO_2 in the form of cellulose (wood) and other metabolic products. This process of removal and storage of CO_2 is called carbon sequestration, performed both by forests and oceans. The removal of carbon from the atmosphere is important because it provides us with clean air, results in oxygen production, provides an energy source for plants, and increases the amount of biomass in a particular area. The production of biomass ensures a continued supply of wood products and other natural resources for human benefit; biomass also provides several benefits to natural ecosystems and animals, and these are indirectly beneficial to humans. Therefore, forest health is important because healthy forests sequester more carbon than unhealthy forests. Knowing this we can consider several questions:

a) What is the current state-of-health of our forests?
b) If our forests are not healthy, what factors are causing them to be unhealthy?
c) What can be done to maintain or improve forest health?

The goal of Forest Watch is to help determine the health of New England forests. In this process we wish to evaluate those factors which cause a decline in forest health. In the Forest Watch program we hope to accomplish this goal by assessing one component of forest health: ozone pollution impacts on our majestic white pine trees.

Healthy Forests Are Important

As alluded to above, natural and anthropogenic factors are known to influence the health of forest trees. Soil factors, for example, determine not only what types of trees grow in an area, but also how well they will grow. Trees dominating wetlands, such as willows, red maples, and black spruce, can tolerate wet, water-logged soils. We all have seen cases where an area has become flooded and the trees have died (e.g., beaver ponds, damming of streams by road construction). This occurs because trees in water-logged soils are suddenly exposed to conditions of low soil oxygen levels. Since most trees are not tolerant of this condition, they die.

Another natural factor, soil mineral composition, will also determine the types of trees growing in a specific area. Red cedar is commonly found in areas where soils have been derived from the weathering of limestone-rich bedrock. On the other hand, since limestone-rich soils tend to be low in minerals such as iron and magnesium, many hardwoods do not grow well in this type of soil; cedars can tolerate these conditions. Other natural factors that can affect tree and forest health include:

- Insects, such as the White Pine Weevil
- Diseases, such as the Dutch Elm Disease
- Drought
- Climate (the long-term trend in weather for an area)
- Damage from severe weather events, such as the ice storm in January, 1998
- Animal damage
- Invasion of non-native species

Human-induced factors are often more important than natural factors to tree and forest health. Air pollution, for example, causes trees and forests to become stressed and unhealthy. The burning of fossil fuels (coal, oil and gasoline products) release CO_2 and other harmful chemicals such as oxides of nitrogen (NO_x) and sulfur (SO_x) into the atmosphere. Even burning wood releases pollutants. These primary pollutants and the secondary products formed as a result of interaction with other environmental factors, can severely impair the health of forests.

There are several other human factors which can affect forests. Some of these factors are listed below, see if you can add to this list:

- Forest fragmentation
- Chemical contamination
- Road salt spray or road salt intrusion into the root zone
- Physical injuries such as logging damage to standing trees
- Incorrect pruning of trees
- Soil compaction
- Assisting in the spread of pathogens
- Introduction of foreign species
- Flooding an area

The Forest Watch program focuses on ground-level ozone and its impact on white pine. Since white pine is sensitive to ozone pollution, we consider white pine a bioindicator species. Ozone produces characteristic foliar (needle) symptoms that can be observed and measured in the lab. The **Tropospheric Ozone and Its Importance** section discusses where ozone comes from and how it impacts sensitive tree/plant species, such as white pine.

While participating in Forest Watch, you and your students will learn how to make meaningful and accurate measurements of needle symptoms and other related variables. These measurements will assist UNH scientists in determining the health of white pines across New England. Through the Forest Watch partnership, participants contribute to on-going UNH research by conducting authentic science and hands-on activities. Additionally, you and your students will begin to understand what can be done to improve both air quality and forest state-of-health by better understanding the cause and effect relationship that exists between specific air pollutants and sensitive forest species.

At this point you may be asking several question, such as:

- How can we tell if a forest (your white pine forest) is healthy?
- What should you and your students look for?
- Once identified, how can indicators of forest health be measured?
- How can forest health be assessed over time?
- And, where do we begin?

These kinds of questions are addressed in the Forest Watch program and, with the help of the Forest Watch Study Guide, you may begin to find some of the answers. The scientific protocols found in the Forest Watch Study Guide provide instruction for accurate, student-generated data that scientists will then be able to use with confidence in their on-going research on forest health. When properly followed, these protocols not only guide you through the steps of acquiring and assessing data on forest health but they also allow for the comparison of data between different sites located across New England. Since the equipment used in Forest Watch is standard and all students will collect their data in the same way, student measurements can be compared with those made by UNH scientists. Soon, you and your students may begin asking your own questions and designing your own research projects to try to answer them.

The information contained in the sections below will help you prepare to answer some of the questions that we have posed above.

Forest Watch Science summarizes the lessons learned to date—it showcases the value of student participation and contributions to scientific research.

What is Forest Watch? provides a detailed overview of the program useful for sharing with colleagues and other teachers interested in participating in Forest Watch, administrators, parents and community members who wish to learn about the types of activities conducted.

From an educational standpoint, Forest Watch students will learn about the process of doing science through hands-on science activities. Learning to do science is like learning to play basketball, it is best done by doing it, not reading about it. Being active participants allows students to "learn by doing," and to develop an ownership in both the process and the products. Through Forest Watch, we all benefit: scientists, students, and teachers. Noel Brown, of the United Nations Environment Program (UNEP) in New York, has stated that "On Spaceship Earth there can be no passengers, only members of the crew." The Forest Watch program is designed for you and your students to be members of the crew.

We welcome you and your students, as together, we come to better understand the health of New England forests, and those factors which impact that health over time.

Forest Watch Science

LESSONS LEARNED

The needle samples that students send to UNH have made a significant contribution to developing a long-term database of needle health as measured through spectral analysis. Access to this continuing long-term database has identified an inverse relationship between yearly ozone levels and spectral indicators of chlorophyll for the same trees, year after year. Participation by several schools across the New England region provides an amazingly large sample (population) size; this has led to the development of a yearly database which clearly reflects annual fluctuations in both white pine state-of-health and local ozone levels. These observations support the hypothesis that variations in ozone levels from year-to-year and across New England can result in variation in white pine state-of-health. The message is now clear: Forest Watch students are helping us improve our understanding of the responsiveness of white pine to factors such as ozone and how white pine state-of-health changes over time.

The examples below expand on how student contributions have improved and documented this understanding.

Change-Over-Time Lessons

In 1992, teachers from eight New Hampshire schools were trained and participated in the Forest Watch program. Six of those original schools have continued to send data for their same five trees for the past seven years, including the spring of 1998. This has provided us the ability to analyze changing state-of-health for a total ,continuous sample size of 30 trees for seven growing seasons. Since needles collected during the spring are needles which grew the previous year, our needle data set spans the years from 1991-1997 (schools who are planning to collect this spring, 1999, will sample needles produced last summer, 1998). This data base is now large enough to allow us to conduct some trend analyses.

It is significant that 75% of the original schools have remained active over the past seven years; the development of a long-term database necessary for assessing the state-of-health of forests over time relies on such a commitment. From these six schools, and other

Figure 1: Map of school and ozone locations for 1991-1997 analysis in Figure 2

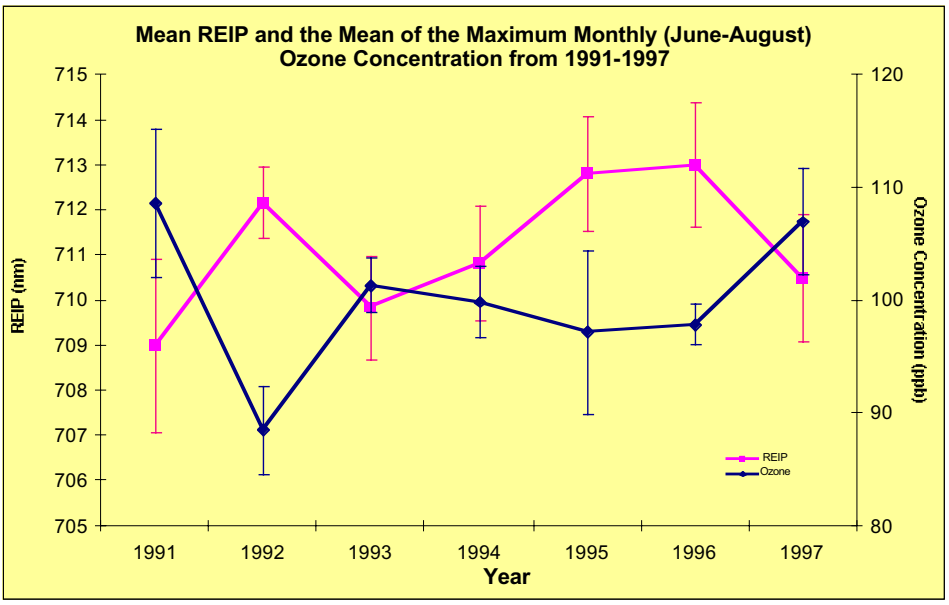


Figure 2: REIP values from six NH schools plotted against ozone from 7 monitoring sites across NH.

participating schools, we are learning how trees react to changes in the environment. The location of each school is presented on the map in Figure 1, along with the locations of ozone monitoring stations which have been in operation during the same time frame.

To help determine if the spectral data that we collect at UNH is related to ozone data collected by the state, we have plotted these data and conducted some preliminary comparisons. From this analysis we can observe the potential effect of ozone on white pine needle health.

Figure 2 shows Red Edge Inflection Point (REIP) plotted against ozone. As you remember, or will learn through participation in Forest Watch, REIP is a spectral measurement made using needle samples sent to UNH. These spectral measurements can be used to infer needle health. Take a minute to study this figure and see if you observe any trends in the data. This graph will be interpreted in detail, but first we will discuss why REIP can be used as a proxy for determining needle health.

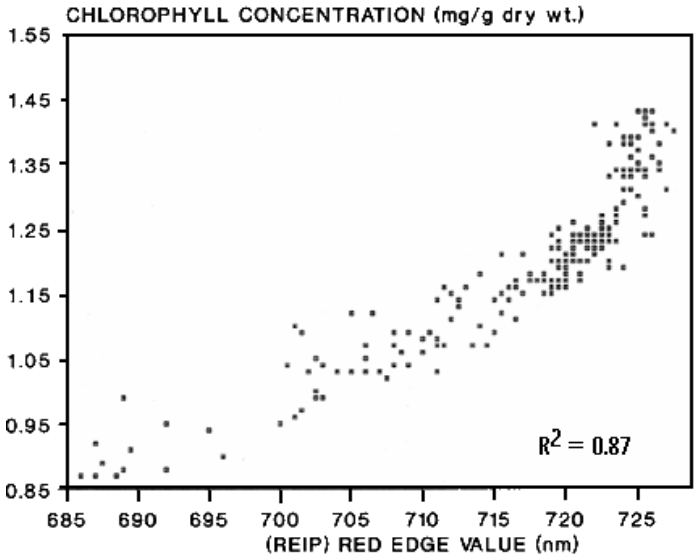


Figure 3: This shows that as the chlorophyll concentration increases, the REIP spectral value also increases—the higher the REIP the healthier the needle.

Interpretation of the data presented in Figure 2 requires some explanation. The Red Edge Inflection Point (REIP) has been closely linked to chlorophyll concentration (see Figure 3); studies have shown that low REIP values are correlated with a loss of chlorophyll. Ozone enters the needle through the stomata and diffuses throughout the intercellular air spaces causing the cellular membranes, including chloroplasts, to degrade. This damage occurs because ozone is a very unstable molecule and is therefore a strong oxidizing agent. The higher the ambient levels of ozone the greater, and faster the damage to mesophyll cells of the needle. Both the ozone concentration level and the duration of that level are important for understanding the damage done at the cellular level. Damage from ozone can occur at high levels of ozone for a short period of time (>100 ppb for 1 hour) or from lower levels of ozone over a longer period (>50 ppb for several hours). The greater the damage the lower the REIP value. It is important to keep in mind, however, that ozone is only one possible cause for reduction in chlorophyll.

Now look back at Figure 2—it plots data from 1991 through 1997, representing student collections made from 1992 to 1998 (1991-1997 needles). The ozone values are an average of the highest monthly value measured from June, July and August, for each year. These values do not take into account the duration of each maximum value, but do represent a way of showing relative differences in ozone levels by year. This is only one possible way to plot the ozone data. The monthly average values for ozone characterizing any given month would be much lower, but the way it is plotted in Figure 2 is a good way to begin REIP vs. ozone comparisons.

Our studies have shown that healthy white pine needles should have a REIP value between 712 nm and 720 nm, whereas a damaged white pine needle will be below 712 nm. By looking at Figure 2, we can see that in some years we have an average REIP value (average of the 30 trees) which is considered healthy and in other years we have a value that we would describe as less-than-healthy. You can also see that the ozone values fluctuate from year to year as well.

The 709 nm REIP value measured in the 1991 needles represents a low level of chlorophyll for those needles. The 712 nm REIP value for the following year was measured from new needles produced in 1992 and collected from the same 30 trees. This indicates a significant improvement in amount of chlorophyll in these new needles. You may think that the difference between 709 and 712 (only 3 nm) is not very significant, but in fact, based on many years of research at UNH on many types of trees (both hardwoods and softwoods), this difference is highly significant and represents a range from low amounts to normal levels of chlorophyll.

The single REIP value given for each year represents an average of 30 trees from around the state: from coastal to well inland and from urban to rural schools. This variation suggests a regional response to some factor or factors which also vary from year to year. You should note that based on REIP values, the trees were healthiest (had the highest REIP values) in 1992, 1995 and 1996, while they were less healthly (had lower REIP values) in 1991, 1993 and 1997. The vertical bars are the standard error for each average and indicate the amount of variation in REIP values from the 30 trees for a given year. The error bar for the 1991 REIP average is longer (ranging from 707.0 to 710.9 nm) than the error bar for 1992 (711.2 to 712.9 nm), indicating that there was more variation in REIP values in 1991 than in 1992; this also suggests that 1992 was a benign year for ozone.

What factors might account for such a dramatic change in the same trees over a seven year period? One factor that could account for such change is exposure to ozone, and this data set supports that view. Based on the data presented above, ozone levels in 1991 (a poor air-quality summer) were considerably worse than those in 1992 (a benign summer). Remember that the needle collections for 1991 needles had low REIP values, while the needles from the same trees, produced one year later (1992) had some of the highest REIP values. The see-saw differences observed between 1991-1997 measurements for both REIP and ozone support the hypothesis that ozone may be a significant factor influencing chlorophyll levels from year to year (especially since they represent a clear inverse relationship). The relative intensity differences in REIP values between years also suggest an ozone influence, since ozone levels for 1993 were high, but not as high as in 1991, and the chlorophyll levels were low in 1993, but not as low as in 1991.

Three very important lessons can be drawn from the Forest Watch data collected between 1992 and 1998:

1. An indicator of state-of-health (REIP) for the same 30 trees can (and does) change from year-to-year;
2. These changes correspond in an inverse way with year-to-year variations in ozone levels for the same region in which the trees occur; and
3. When ozone levels are low, state-of-health improves, suggesting that white pine is sensitive to variations in ozone exposure.

Based on these data, we can't say for certain that ozone has caused the change in REIP values, but we can say that a strong inverse relationship can be seen between inter-annual variations in ozone levels and pine needle REIP values in samples collected by Forest Watch students in New Hampshire.

Another important lesson can also be derived from the student data collected between 1992 and 1998; the resiliency of white pine to improved ozone conditions is clearly evident. It is important to recognize that factors other than ozone may play a significant role in changing state-of-health in white pine. The climatic conditions which promote the formation of elevated levels of tropospheric ozone (hot, sunny summer months), coupled with high levels of NO_2 and other components of automobile exhaust, may also be factors affecting white pine state-of-health. Previous research at UNH and elsewhere has indicated that although other factors are important, ozone levels have been shown to be the single-most important factor relative to production of wood in white pine. Student observations of needle symptoms characteristic of ozone damage (chlorotic mottle and tip necrosis), along with other forest stand factors (canopy closure, extent of ground cover, diameter growth rate, etc.) may be used to evaluate the relative impacts of such factors in the year-to-year variations seen in the student branch collections.

None of these observations would have been possible without the measurements of the same trees over the six-year period. The Forest Watch student and scientist partnership is helping us to better understand ozone's impact on white pine health. Each year the Forest Watch database grows, as does the number of schools involved, resulting in the ability to conduct studies on the changes in state-of-health, both over time and regionally, of white pine. Our findings are supporting the hypothesis that changes in white pine health may be linked to changes in air quality.

If you have been involved with Forest Watch previously, we thank you for your continued participation. If you are new to Forest Watch, we believe that your students will find this program a valuable experience in learning science. We hope that you will find these materials helpful for implementing the program in your classroom and, as always, we are open to your comments and suggestions to help us improve the effort to understanding the health of our forests.

What Is Forest Watch?

A BRIEF INTRODUCTION

Forest Watch is a New England-wide environmental education program designed to introduce both teachers and their students to field, laboratory, and satellite data analysis methods for assessing the state-of-health of local forest stands. Forest Watch provides workshops which are designed to help K-12 and post-secondary teachers introduce students to selected hands-on techniques, based on UNH research methods, for evaluating the health of white pine (*Pinus strobus*)—a bioindicator for ground-level ozone. Through the Forest Watch program, students become actively involved in doing meaningful scientific research, and in the process, collect and compile data useful to UNH researchers in their on-going regional survey of forest health. This program was introduced in 1992 to six New Hampshire schools; it is now in the seventh year, with participating schools from New Hampshire, Maine, Vermont, Massachusetts, Connecticut, Rhode Island and Maryland.

The Forest Watch program grew out of research conducted by Dr. Barry Rock of Complex Systems Research Center at the University of New Hampshire (UNH); his research in the early 1990s focused on assessing the relative sensitivity of several bioindicator species to controlled levels of ozone exposure in order characterize responses at the visual, physiological, and spectral levels. Rock and his associates demonstrated that white pine needle response to elevated levels of ozone above 80 ppb (parts per billion) occurred within one hour of exposure. This work led to the identification of many other potential bioindicator species for ozone exposure, including black cherry, dogbane, milkweed, bigleaf aster and white ash. White pine was selected for study in the Forest Watch program because it was shown to be sensitive to ozone exposure, it occurs commonly across New England (often near schools), and it exhibits characteristic needle symptoms year-round. All of the other bioindicators are deciduous and exhibit their symptoms only during the mid-summer (late July to early August), when schools are not in session.

Students participate in three types of activities in Forest Watch, each patterned after activities conducted by professional environmental scientists. The activities include:

1) forest stand assessment,
2) laboratory-based assessment of damage symptoms, and
3) image processing/data analysis.

Each activity is designed to provide results which are both quantitative and meaningful at a variety of scales, from the cellular to the forest stand level and up to a regional scale. Students' field and laboratory measurements are sent to the University of New Hampshire where they are analyzed and integrated into the regional white pine study. Students also send a duplicate set of needle samples to UNH that are analyzed with a field reflectance spectrometer for a state-of-health assessment. Each year a compilation of Forest Watch results is published consisting of all student-generated field and laboratory measurements and the UNH-collected spectral reflectance data.

Forest stand assessment activities introduce students to ground truth activities designed to provide data on forest stand parameters such as species composition, tree size, canopy closure, and ground cover. In order to conduct these measurements, students set up a long-term sampling plot and permanently tag five white pines trees. They estimate tree height using hand-made clinometers to determine the angular distance to the top of each tree from a known baseline distance; they determine tree diameter at breast height (DBH) by measuring the circumference at 1.35 m above the ground and dividing by *pi*; they use a hand-made canopy densiometer to determine percent canopy closure; and they measure percent green ground cover. All of these activities produce data useful in comparing each school's study site with other schools' sites within the Forest Watch study area.

Students conduct laboratory-based analyses of ozone damage on white pine foliage by measuring needle length for thirty current-year needles and identifying percent occurrence of foliar symptoms characteristic of ozone injury (chlorotic mottle, tip necrosis). They also

record the number of years of needles retained; healthy white pine may keep up to three years of needles, but will shed older needles if they are not productive. The fewer the number of years of needles, the less healthy the tree. In some of the more heavily-polluted areas of New England (along the seacoast, near big cities), only one or two years of needles are retained (based on student data).

Students are also introduced to hand-sectioning of needles for study under the microscope. In this optional activity, students section both healthy and damaged portions of needles, and investigate the cellular-level basis for the visual symptoms (chlorotic mottle, tip necrosis). Two other optional activities, extraction of chlorophyll pigments and determination of wet weight/ dry weight, are conducted by several schools.

Forest Watch combines space-age technology with old fashion biology. Field and laboratory activities are integrated with image processing activities to introduce students to the use of Earth-orbiting satellites as environmental monitoring tools. Students use *MultiSpec* image processing software to display and analyze local Landsat data, thus allowing students to see how their study plot can be used to study forest health on a regional perspective.

Since students measure the same trees each year, growth rates and estimations of carbon sequestration will be monitored and characterized relative to the ozone symptomology over time. At present, over 120 schools are involved in the Forest Watch program, and the student-derived datasets have already made a very significant contribution to improving our understanding of variations in forest health and summer ozone levels (see **Lessons Learned**).

The Forest Watch program is exciting because it creates a genuine partnership between the scientific research community and the students and teachers in schools around New England. The measurements made by students, using carefully-designed Forest Watch protocols, provide researchers with accurate and meaningful data. The students become active participants in the scientific process. At the same time, the scientists provide guidance and direction to teachers and their students, offering a source of expertise on regionally-important environmental topics. A mutually beneficial and lasting alliance is thus established between the university research laboratory and the classroom. Forest Watch uses authentic science and innovative experience-based education to study our home planet and the impacts of air pollution on regional forests.

For more information on *Forest Watch*, contact either Dr. Barry Rock (Program Director) or Mr. Shannon Spencer (Program Coordinator) at forestwatch@unh.edu or (603) 862-1792.

Forest Watch Goals and Objectives

TO ASSESS THE HEALTH OF NEW ENGLAND FORESTS THROUGH A STUDENT AND SCIENTIST RESEARCH PARTNERSHIP.

Forest Watch is:

- *Hands-on/minds-on research*
- *Authentic science*
- *An interdisciplinary approach to learning*
- *A true student and scientist partnership*
- *An opportunity for students to become active contributors to an on-going research project*

Educational Objectives

1. Engage students in authentic science by participation in a Student-Scientist Partnership.
2. Provide hands-on measurement activities which use scientific and technological tools in the classroom to contribute meaningful data to research scientists.
3. Enhance higher-order thinking skills by introducing students to local, regional and global issues.
4. Develop data analysis and communication skills.
5. Provide integrated activities that encourage a teamwork environment.
6. Offer pedagogical strategies to meet state and national standards in science and mathematics.
7. Affect a positive change in student attitudes and aptitudes for science and mathematics.

Science Objectives

1. Determine parameters needed to assess white pine health at both the local and regional level.
2. Correlate student physical measurements with spectral measurements made of needles.
3. Compare physical/site parameters and spectral measurements with variations in ground-level ozone data on both a regional and annual basis.
4. Characterize the use of white pine as a bioindicator for use in future research studies of the health of New England forests.
5. Develop a long-term database of ecologically important variables for use in future research studies of the health of New England forests.
6. Assess health using a site index.

Using the Forest Watch Teacher's Guide

WELCOME TO FOREST WATCH!

The Forest Watch Teacher's Guide is divided into three separate sections, each in its own notebook:

Introductory and Mathematics Support Activities

The Introductory and Mathematics Support Activities include preliminary exercises that introduce students to the Forest Watch concepts. These activities may be used just before the time of needle collection or as preview activities at any convenient time earlier in the school year. Contained in this set of materials you will find several math activities that will help you and your students take a closer look at your data so that you can assess it carefully and compare and make conclusions about what your data mean.

Forest Watch Protocols

In the Forest Watch program, a distinction is made between a scientific protocol and its support activities. This is because of the way in which the data and information gathered in the protocols will be used by researchers at UNH. In scientific research, science protocols are exact procedures that have been developed to answer specific questions. Protocols are necessary for the exact duplication, verification and validation of experimental results. They are meant to be performed in exactly the same way by every student in every school. Protocol data may be shared among scientists, students and teachers across many schools, states, regions and even countries. The interpretation of the data gathered from the protocol assumes that each step was followed carefully and accurately. Data collected in the required protocols should be summarized on the Master Data sheet or Excel spreadsheet and sent to the University of New Hampshire. Data from activities and optional protocols can be kept in a journal at school for future study and do not need to be sent to the University.

There are five required and two optional protocol sections contained in the Protocol notebook. Protocols consist of information and precise directions for the data gathering and measurement portion of the study. Each of the seven protocol sections begins with a table of contents that provides an overview of the protocols and other activities in that group. Protocols, listed first, contain student and teacher resources. Student resources include background information, the protocol itself and questions for discussion. Teacher resources include additional information such as guiding questions, management suggestions and an answer key. The guiding questions are designed to help students relate the exercise to the overarching theme of forest health assessment through the use of the bioindicator white pine. In this way, a unifying thread runs throughout the entire program.

Several of the protocol sections have optional support activities that are designed to enhance student understanding of the particular topic covered in that section. Some of the activities are to be used before, while others are best used as a follow-up to the main investigation.

Forest Watch Extensions

The last part of the Teacher's Guide contains a set of exercises that help illustrate how remote sensing, through the use of satellite images, can be used as a tool for interpreting forest health on a regional scale.

Forest Watch Extensions include a map, photo and satellite image interpretation activity as well as a remote sensing primer and activities, which utilize a computer program, called MultiSpec. Among its many uses, remote sensing tools can aid in the study of large-scale ecosystems for forest damage symptoms. Some teachers may choose to use the MultiSpec activities as an introduction to the Forest Watch project. Alternatively, MultiSpec can be used after the Forest Watch study has been completed to help the student gain a regional as well as a global perspective on forest health assessment.

These exercises and a satellite image of the area around your school and study site are available after you attend a special workshop designed to train teachers specifi-

cally in these topics. If you would like information about workshops on remote sensing topics, please call or email Forest Watch at the University of New Hampshire.

The Introductory and Mathematics Activities and the Protocols have been arranged in the binders in a suggested sequence for completion. The actual sequence that you choose may vary depending on access to materials to be used in the field or lab, weather conditions on a particular day, the appropriateness of an activity for a certain group of students and so on.

Tropospheric Ozone and Its Importance

In an effort to better understand the effects of tropospheric ozone on the health of animals and plants the following background information on tropospheric ozone is provided.

The air we breathe is clearly one of our most vital resources. Ambient ozone pollution in urban and regional air has proven to be one of this country's most pervasive and difficult to solve environmental problems. Despite more than two decades of massive and costly efforts to bring this problem under control, ozone pollution continues to exceed national ambient air quality standards in areas where 65-125 million people live. Despite significant progress in reducing the burden of certain types of air pollution in the United States (i.e., particulates and sulfur dioxide), we are confronted with new, increasingly complex federal and state air pollution regulations in recent years. The U.S. Clean Air Act Amendments of 1990, and some of the recent air quality regulations established in California, are controversial because they are costly to implement and impinge on how we may live our lives in certain areas of the country. On the other hand, robust data on the health and environmental benefits of our increasingly strict air pollution regulations are difficult to define.

Members of Congress, the business community, and many others are asking a number of difficult questions:

- Why hasn't more progress been made in reducing the ozone component of smog during the past several decades of regulation, emissions controls and research? What have we learned, and what will the Clean Air Act Amendments of 1990 do, that should give us confidence that additional regulations and investment will produce significant health and environmental benefits?
- Exactly how serious is the problem of ozone pollution in the lower atmosphere? In some locations the health standards are only exceeded during a few hours or days each summer. In this era of budget deficits and numerous other social and economic problems, what priority should be given to investing additional resources on the tropospheric ozone pollution problem?
- Finally, all of this talk about "bad" ozone in the lower atmosphere, and "good" ozone in the stratosphere is confusing: If we lose some high altitude ozone due to stratospheric ozone depletion, can it be replaced by the enhanced ozone which is produced by tropospheric pollution?

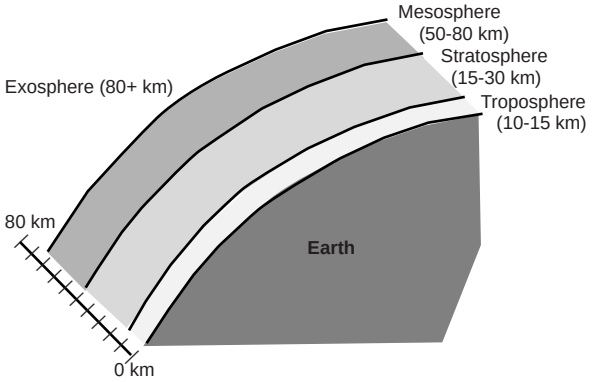
We will discuss the current scientific evidence which bears on these and other questions that relate to what we know, and don't know, about the ozone pollution problem in the air we breathe. We will first consider what ozone is, and how it is produced in, and removed from, the lower atmosphere. The next step will be to understand where and when ozone pollution is a problem. We will also review what is known about the health and environmental effects of elevated levels of near-surface ozone.

What we will find at the end of this review of the scientific data on ozone is that the exact costs and benefits of ozone pollution control cannot be determined by scientific study alone. This problem, and many other environmental issues we will confront such as the production of greenhouse gases and genetically engineered organisms, will require some degree of subjective analysis by citizens. The best possible analysis and decision-making, however, occur when the existing scientific understanding of a problem like ozone pollution is clearly communicated to the public.

What is the difference between tropospheric ozone and stratospheric ozone?

Ozone (O_3) is a gas molecule which is produced naturally in trace amounts in the earth's atmosphere. A molecule of ozone is composed of three oxygen atoms; the third oxygen makes the ozone molecule highly reactive. About 90-95 percent of the earth's ozone is found in the stratosphere (10-25 km above the earth) where it plays a critical role in absorbing harmful ultraviolet (UV) radiation, which can damage living systems. This stratospheric ozone is often referred to as the *Good Ozone*. Ozone depletion in the stratosphere, which results in the "ozone hole," is a distinct problem from tropospheric ozone pollution. The "ozone hole" results from the production and use of man-made chlorofluorocarbons (CFCs) and other halo-carbons.

Stratospheric ozone depletion allows increased UV radiation to reach the Earth's surface thus enhancing the potential for skin cancers, cataracts and plant damage.



On the other hand, tropospheric ozone is *Bad Ozone* because it is a source of pollution in the air we breathe. This air pollution can have significant negative impacts on animal and plant life, especially in higher altitude regions where some plants and animals are bathed in a chronic mist of high levels of ozone—hiking in the White Mountains can actually be bad for you! Unlike the "ozone hole," tropospheric ozone pollution (also called ground-level ozone) is a byproduct of the burning of fossil fuels in industry, energy production and motor vehicles.

For most of the earth's history, ozone was present at such small concentrations in ground-level air that it did no harm. The highest natural concentrations of ozone occurred in the vicinity of a thunderstorm, or on a mountain top. These enhancements result from the temporary penetration of stratospheric air to lower altitudes and from ozone production by intense electrical fields. Typically, the range of ozone concentrations in unpolluted, ground-level air will vary from 0 to 30 parts per billion.

Today, if we go jogging in the afternoon on a hot, hazy summer day in Rye, New Hampshire or Bar Harbor, Maine, we may find ourselves breathing air with 80 to 200 parts per billion ozone. High concentrations of ground-level ozone in places like New Hampshire and Maine may surprise you. These locations are vacation spots; places where people go to escape the crowds and pollution expected in Washington, D.C., Philadelphia, New York, or Boston.

The scientific community has strong reason to believe that concentrations of ozone in near-surface air are increasing over large areas of the earth associated with, and downwind of, human activities. For decades after the first unexpectedly high concentrations of ozone were reported in rural areas, their origin was attributed by most atmospheric scientists to incursions of ozone-rich stratospheric air. Twenty-five years of well-established scientific evidence, however, points to a "pollution" origin for much of the enhancement of near-surface ozone.

How is ozone pollution produced in the air we breathe?

An important aspect of the tropospheric ozone problem is why it forms in and downwind of large cities. On warm, sunny days, emissions to the atmosphere of nitrogen oxides (NO_x) and volatile organic compounds (VOCs) can react to produce ozone concentrations as high as 200 to 400 parts per billion compared to concentrations of 20 to 40 parts per billion on a cloudy and windy day at the same location. Such production of ozone is called photochemical air pollution; it was first studied in Los Angeles and surrounding areas in the 1940's, when vegetable crops began to show damage that could not be related to insects or disease.

Volatile organic compounds, also known as hydrocarbons, are primarily produced by cars and gasoline-burning engines. Additionally, VOCs come from consumer products such as paints, insecticides and cleaners, as well as industrial solvents and chemical manufacturing. Nitrogen oxides come primarily from large industrial activities (energy production) and automobiles, that is, from the burning of fossil fuels. Interestingly, there are natural sources of these precursors to ozone as well!

The details of the atmospheric chemistry of tropospheric ozone formation are very complex. For our purposes, we can simplify the details of photochemical ozone production to a five-step process.

1. In the first step, reactive VOCs, represented as RH (reactive hydrocarbons), react with hydroxyl radicals (OH), the primary oxidant in the atmosphere, to form reactive organic radicals (R):

   $$RH + OH \text{ Æ } R + H_2O$$

2. Organic radicals combine with atmospheric oxygen (O_2) to form gases called peroxy radicals (RO_2):

   $$\mathbf{R + O_2 \rightarrow RO_2}$$

3. The peroxy radicals react with nitric oxide (NO) to form nitrogen dioxide (NO_2):

   $$\mathbf{RO_2 + NO \rightarrow NO_2 + RO}$$

4. Nitrogen dioxide is broken apart (photodissociated) by near-ultraviolet solar radiation (hv) to produce an oxygen atom, and to reform nitric oxide:

   $$\mathbf{NO_2 + hv \rightarrow NO + O}$$

5. The oxygen atom is reactive and combines with molecular oxygen to form ozone:

$$O + O_2 \rightarrow O_3$$

Ozone is subsequently removed from the lower atmosphere by deposition to the surface, or by a two step photodissociation process in the presence of near-ultraviolet solar radiation. The photodissociation of ozone produces an unstable, excited form of the oxygen atom, $O(^1D)$:

$$O_3 + hv \rightarrow O_2 + O(^1D)$$

The $O(^1D)$ rapidly reacts with water vapor (H_2O) to form two OH radicals:

$$O(^1D) + H_2O \rightarrow 2OH$$

We can see that the reactions that photochemically destroy ozone produce two hydroxyl radicals which start the process of ozone formation. The combined processes are a chain reaction. With the right meteorological conditions (sunny, warm, and humid), and with enough NO_x and VOCs (RH) in the atmosphere, the chain reactions listed above can lead to unhealthy concentrations of ozone pollution.

A change in weather from warm, sunny conditions to cooler, rainy days will also inhibit ozone formation. The clouds and rain will remove reactants necessary for ozone formation. For example, nitrogen oxides can be converted to nitric acid which is very soluble in cloud droplets. The scavenging of nitric acid by clouds and precipitation leads to another pollutant—acid rain and fog.

Where do the pollutants that produce ozone come from?

We have established that NO_x and VOCs are critical to photochemical ozone production. A first step in studying pollution control options is to conduct an inventory of emission sources. An emissions inventory is an extremely complicated and difficult task for a large industrial country like the U.S. However, in the case of NO_x and VOCs, a detailed study was conducted as part of a National Acid Precipitation Assessment Program. This study showed that the major categories of NO_x emissions related to human activities are:

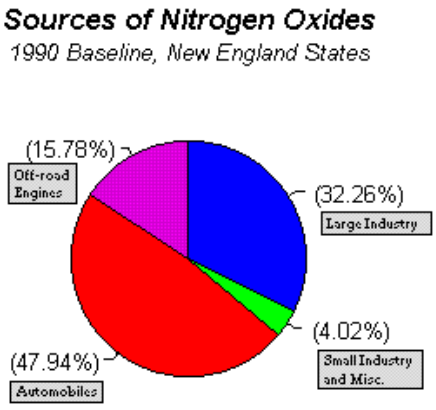
a) motor vehicles,
b) production of electricity,
c) industrial processes.

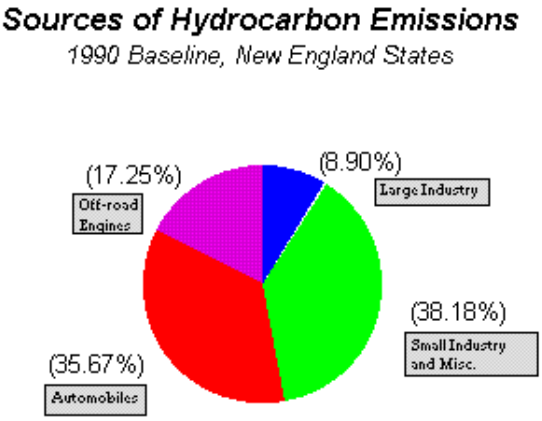
Processes involving the combustion of coal, oil, and natural gas are the primary source of NO_x. We can see that the activities which produce NO_x are fundamental to our lifestyle and economy. Emission control technologies for NO_x are relatively expensive, so early efforts to regulate emissions of ozone precursors focused on controlling VOCs.

Motor vehicles also play a major role as a source of VOCs. Cars and light-duty trucks are the dominant contributors. Solvent emissions, which are distributed across a broad group of industrial sources also contribute a significant percentage. In contrast to NO_x, the production of electricity (large industry) is a very minor source of VOCs.

Evaporation of fuels (such as occurs when filling your car's gas tank at the gas station) and incomplete combustion are the primary sources of VOCs emitted by automobiles. The magnitude of the emissions will depend on factors like ambient temperature, gasoline volatility, engine type, and emissions control system design and operation. It is obvious that all these variables can make estimating national emissions a challenge and some studies claim that the original prediction model underestimates VOC emissions by as much as 30 percent.

The use of organic solvents in the dry cleaning industry, metal degreasing, asphalt paving, and a variety of





manufacturing activities contribute to VOC emissions. Surface coating-related industries involving painting and printing are also sources of VOCs.

What are some natural sources of VOCs? Do trees pollute?!

In 1960 Dr. F.W. Went at Washington State University first proposed that natural emissions of VOCs from trees and other vegetation could have a significant effect on the chemistry of the atmosphere. Went and a number of other researchers have measured VOC emission rates from a wide variety of plants. From these studies it was suspected that VOC emissions from vegetation could be involved in ozone chemistry; however, the quantitative role of natural VOCs in urban photochemical smog has only recently become sufficiently well understood to impact environmental policy and regulation.

An important demonstration of the role natural emissions can play in determining ozone levels was conducted by Dr. William Chameides and co-workers at the Georgia Institute of Technology. They were able to show that for the Atlanta area, the emission of VOCs from vegetation could contribute significantly to ozone production during photochemical smog events. The implications of their study pose a serious challenge to the primary emphasis placed by regulators on VOC emissions reduction as a strategy for ozone pollution abatement.

In areas with significant vegetation it is likely the NO_x controls will be a more effective approach to ozone abatement than VOC controls. However, the cost-effectiveness of NO_x versus VOC controls remains to be resolved. With the new understanding of the potentially important role of natural VOCs (which contribute about eight times as much as human induced sources) in photochemical ozone formation, and improved emissions inventory data, it is reasonable to expect that computer modeling of specific cities and regions will have to be used to develop unique local control strategies.

How does the weather affect ozone pollution?

Most of us still remember the summer of 1988—record heat, humidity, and smog. Ozone accumulates in an area when there are high temperatures and stagnant air. In meteorological terminology major episodes of photochemical ozone pollution are typically associated with slow-moving, high pressure systems. The eastern United States is particularly susceptible to ozone episodes during summer months when a “Bermuda high” dominates the weather for days to weeks. During these periods there is widespread sinking of air through most of the troposphere in the region of high pressure. The falling of air from aloft creates an inversion of the normal temperature profile in the atmosphere. The subsiding air is warmed during descent resulting in a stable atmospheric condition with relatively cooler air at ground level and slightly warmer air above.

During an inversion, the cooler, higher density air near the surface does not mix with warmer air aloft. Because winds associated with a high pressure system are generally light, pollutants accumulate in the region under the influence of high pressure. The most serious ozone episodes in the eastern U.S. have occurred when Bermuda high conditions develop around the time of the summer solstice. This is the time with the greatest amount of daylight and when solar radiation is most direct. High ozone episodes are often terminated by the passage of a front which brings cooler, cleaner air or precipitation into the region.

The infamous smog episodes in the Los Angeles basin illustrate how meteorology can interact with a unique landform to produce a pollution-prone region. Because the weather in that area is dominated by a persistent Pacific high and the mountains surrounding the Los Angeles basin enhance the sinking motion of the upper atmosphere, polluted air becomes trapped at low altitudes below the temperature inversion. These factors, combined with high emissions, make the environment ideally suited for ozone pollution.

What are the health effects of ozone pollution?

The health risks of ozone pollution are relatively easy to define for the portion of the population who are uniquely sensitive, although in many of these cases ozone may only be a contributing factor to acute stress and not the single cause. For most individuals ozone pollution is a chronic stress—producing what is currently judged to be a subtle reduction in physiological performance. Another important consideration in weighing the health risks of ozone, however, is the involuntary nature of the exposure. When compared to more hazardous, voluntary risks like skiing, mountain climbing, or riding a motorcycle, most people place a higher priority on controlling involuntary hazards.

The discovery of health effects due to exposure to outdoor ozone pollution was first reported in 1967, when high school athletes in California were shown to be more likely to exhibit lower performance on high exposure days. An important study by A.J. DeLucia and W.C. Adams in 1977 showed that asthmatic adults who were exercising and were exposed for one hour to 150 parts per billion ozone in a laboratory test chamber had increased coughing and wheezing, and decreased

pulmonary function. Subsequently, there have been numerous studies on healthy young men and women which have detected a response, usually in lung tissue performance or condition, at ozone exposures as low as 80 parts per billion.

There are appropriate measures that people can take to minimize their exposure to unhealthy levels of ozone. Because ozone is a secondary pollutant produced by photochemical reactions, the higher concentrations typically occur between noon and 10 p.m. The enhanced concentrations in late afternoon and evening are most important in areas downwind of urban centers, due to downwind transport of ozone formed hours earlier. During the night, ozone is no longer being produced, and molecules in near-surface air which come in contact with vegetation, soil, or other surfaces will react and be consumed. Consequently, early morning hours are generally the period of minimum ozone concentrations during periods of hot, stagnant summer weather.

What are the ozone-related symptoms on eastern white pine?

Eastern white pine trees affected by ozone or ozone-related pollution complexes may display two types of injury symptoms on their foliage. These symptoms are described as “ozone injury” and “chlorotic dwarf.” An ozone-induced tip necrosis on the new needles of eastern white pine was first called “emergence tipburn” and later, symptoms ranging from chlorotic flecks through chlorotic mottling, to a severe tip necrosis were labeled “ozone injury.” Damage results from ozone entering the needles through the stomata. Inside the needle, the reactive ozone attacks cell membranes, decreasing the efficiency of membrane function. This increases the respiration rate as the membranes are repaired. Because energy is needed for the increased respiration rate, the rate of photosynthesis is greatly decreased. This, in turn, reduces carbon accumulation in the plant tissue resulting in weak or stunted growth.

The initial macroscopic symptoms of ozone injury are minute, silver flecks radiating from the stomata of current-year needles. These tiny silvery flecks, which are best seen under magnification, develop into larger chlorotic mottle visible to the naked eye. Semi-mature tissue is the most seriously affected portion of the needle, but the immature and the mature tissues are often simultaneously affected. Chlorotic flecks may develop into pink lesions and bands followed by a distally spreading, orange-red necrosis which may take 1-2 weeks to reach the needle. On less sensitive trees only chlorotic flecks and mottling may occur, whereas severe needle tip necrosis occurs on more sensitive trees. Microscopically, mesophyll cells adjacent to the stomata are the first to be affected, and endodermis and stele are the last to be affected. New, rapidly growing needles (from about 1 week after emergence until 6 weeks of age) are most sensitive. Normally, 3 years of needle ages occur on eastern white pine. However, on ozone-sensitive trees, the older needles become prematurely senescent and discolored, being cast by mid-summer, leaving only the current-year needles.

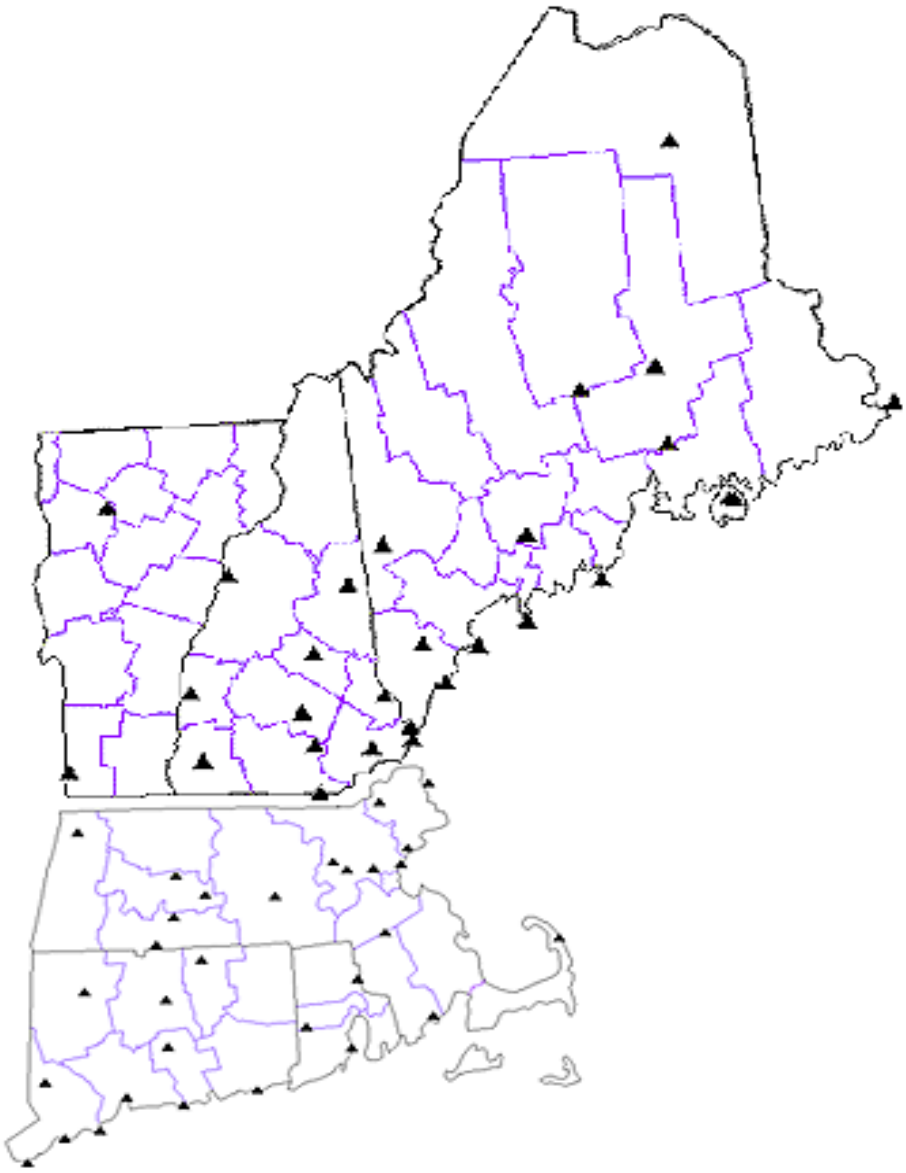
The chlorotic dwarf disease affecting eastern white pine has been observed for 70 years. Etiological studies, studies of cause, have demonstrated that air pollutants are the primary causal agents of the disease. On genetically susceptible trees, the current-year needles emerge normally, but not long after they have attained some growth, their natural green color becomes spotted with chlorotic flecks and mottling. The older foliage turns prematurely yellow and is shed before the current-year needles reach full development. In final stages, particularly following a drought, the affected current-year needles may develop tipburn.

The disease occurs most commonly on young pines in large homogeneous stands, and severely affected trees usually succumb before they reach 15 years of age. White pines vary considerably in their susceptibility to chlorotic dwarf, with less sensitive trees exhibiting mild mottling symptoms on near-normal length needles, whereas more sensitive trees exhibit severe stunting of all plant parts and yellow mottled, possibly curled, current-year needles.

How is ozone monitored?

Ozone, and other pollutants that EPA has designated as criteria pollutants, is monitored at various sites around the country. Typically each state has a number of monitoring sites located in areas which are likely to be impacted by certain pollutants and which may adversely effect people living or working nearby. Each monitoring station generally consists of a small trailer with air sampling equipment, precipitation collection devices and other meteorological instruments that collect information on specific problem pollutants for that area. Not all variables are measured at each station.

The map below shows the location of the stations which measure ozone concentrations in the air across New England. Since ozone is formed from primary pollutants such as VOCs and NOx and also requires high temperatures and plentiful ultraviolet radiation, ozone is generally only measured from late spring to early fall. This is the period when conditions are right for the formation of ozone in New England. Monitoring stations are typically run by air quality agencies within each state, in association with the EPA. The actual

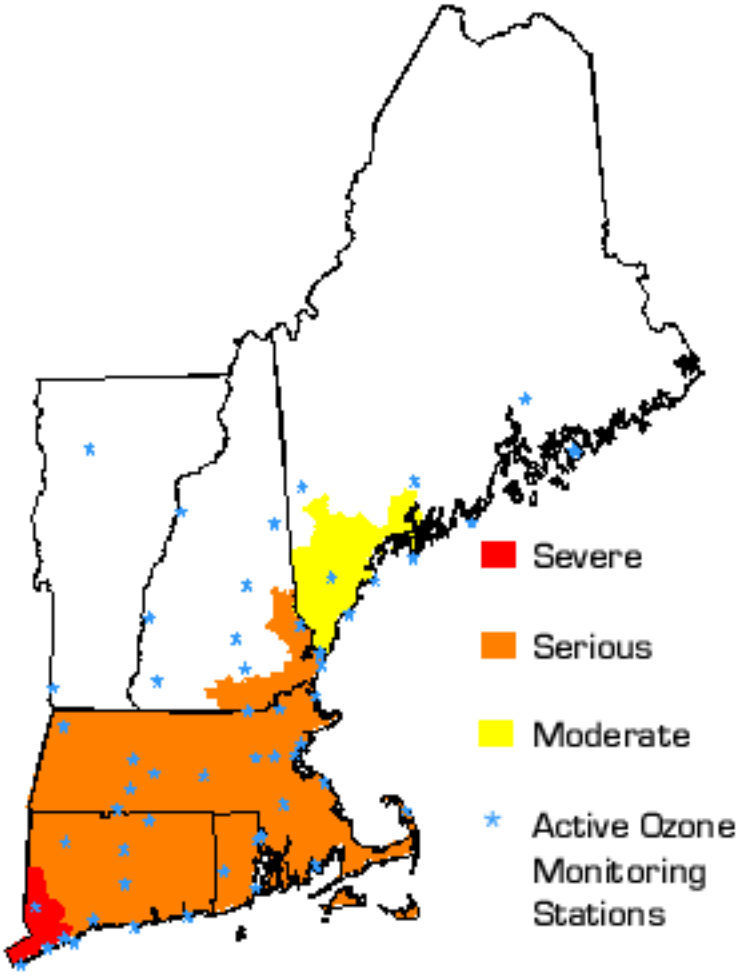


Ozone Monitoring Sites in New England

process of measuring the concentration of ozone in the air samples is fairly complicated but involves gas chromatography. The data are submitted to the EPA and are publicly available. The data are generally collected continuously but are often reported on an hourly, daily or monthly average—depending on the intended use of the data.

Once EPA has the data, they monitor changes in ozone concentrations over time to determine if specific geographic regions have unhealthy levels. Areas which do not meet the EPA NAAQS standards (discussed above and below) over a 3-year period are considered to be in "nonattainment." Nonattainment areas become subjected to certain political and regulatory restrictions and are required to make corrective actions to reduce the ozone levels. The following maps and discussion show areas of both New England and the US which were in nonattainment as of 1997, under the old EPA NAAQS standard of 120 ppb.

Ozone Non-attainment Areas in New England



Source: EPA Region 1 Web site: http://www.epa.gov/region01

EPA has set National Ambient Air Quality Standards (NAAQS) for ground-level ozone. The map above shows the areas in New England that are presently designated as "nonattainment" for the one-hour ozone standard of 0.12 parts per million (ppm). Nonattainment areas are those areas which experience ground-level ozone concentrations exceeding the 1-hour ozone standard more than once per year (based on a 3 year average). Areas designated as nonattainment are classified as marginal, moderate, serious, severe, or extreme depending upon the severity of the air quality problem at the time of the Clean Air Act of 1990 passage.

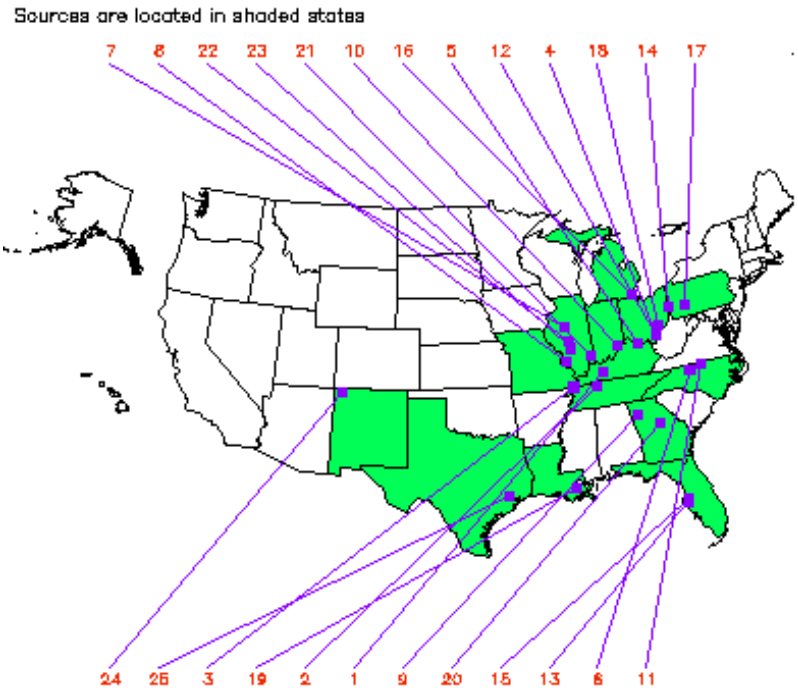
In July of 1997, EPA issued new National Ambient Air Quality Standards for ground-level ozone. The new standard is set at 0.08 ppm averaged over 8 hours. Areas are not attaining the new standard if the 3-year average of the annual 4th highest daily maximum 8-hour ozone concentration exceeds 0.08 ppm. EPA will designate areas as nonattainment for the new ozone standard in the year 2000, based on the most recently available three years of air quality data at that time (e.g., 1997-1999).

The figure on the next page shows the nonattainment areas for the whole country as of December, 1997. These are areas which are currently in violation of the 1-hour EPA NAAQS. The last two figures show the distribution of the top 25 sources of both nitrogen dioxide and volatile organic compounds, these sources are stationary industrial/energy production facilities which have significant output of ozone precursors—chemicals which react with sunlight to form ozone (see previous discussion on ozone formation). Automobiles and other small, mobile engines are also a significant source of pollutants, but are not shown on these maps.

Nonattainment Areas for the Contiguous United States as of December, 1997

Source: adapted from AIRS Data Web site: http://www.epa.gov/airs/

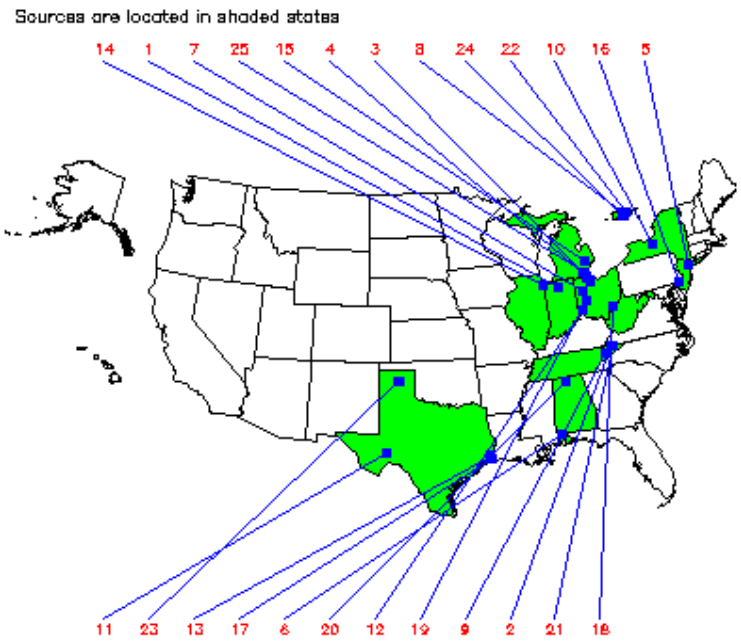
The Top 25 Stationary Nitrogen Dioxide Pollution Sources



	Estimated Annual Emissions Thousands of Tons	(emis.inv. year)	
1	113	(96)	TVA
2	107	(93)	TVA Cumberland Steam PLA
3	94.7	(95)	Associated Electric Coop
4	86.7	(90)	General James M. Gaven P
5	76.9	(90)	James M. Stuart Elec Gene
6	74.6	(95)	Duke Power Co. Belews C
7	72.6	(97)	Com Ed-Powerton General
8	63.1	(97)	Illinois Power co.-Baldw
9	63.1	(90)	GA Power Co Bowen Stm El
10	59.5	(97)	Indiana-Kentucky Electri
11	59.4	(97)	CP&L-Roxboro Steam Elect
12	57.5	(90)	Kyger Creek Station Ohio
13	57.0	(96)	TECO
14	54.2	(90)	Ohio Edison Company W H
15	51.2	(96)	Tampa Electric Company
16	47.9	(97)	Detroit Edison Co
17	48.3	(95)	GLU Gen Corp/Homer City
18	45.4	(90)	Muskingum River Plan
19	44.3	(95)	Bunce Corp
20	43.3	(90)	GA Power Co Branch STM E
21	40.2	(96)	PSI Energy-Gibson
22	39.0	(97)	Com Ed-Kincaid Generat
23	38.8	(97)	Central Illiois PUblic
24	38.8	(98)	Public Serv Co NM/San Ju
25	38.1	(96)	Houston Industries Inc.

Source: adapted from Airs Data Web site: http://www.epa.gov/airs/

The Top 25 Stationary Volatile Organic Compounds Pollution Sources



Estimated Annual Emissions
Thousands of Tons (emis.inv. year)

1	19.2	(97)	Indiana Heat Transfer Co
2	17.8	(93)	Tenn Eastman Co
3	17.2	(90)	Chemi-Trol Chemical Co
4	15.3	(90)	BP Oil Company
5	14.3	(90)	Allied Extruders Inc
6	14.2	(95)	Courtaulds Fibers Inc
7	12.6	(90)	BP Chemicals Inc.
8	12.2	(92)	Fantastic Dry Cleaners
9	11.9	(92)	Lenzing Fibers Corporati
10	10.2	(90)	TRW Trans Elec Div
11	9.79	(96)	Western Gas Resources
12	9.01	(90)	AK Steel Corporaton
13	8.72	(96)	Mobil Oil Company
14	8.52	(97)	Acme Steel Company
15	8.32	(97)	Mantex Corp
16	7.55	(90)	Air Products and Chemica
17	7.73	(98)	Clark Refining & Marketi
18	7.60	(96)	Dupont Washington Works
19	7.49	(90)	Impression Coatings
20	7.08	(98)	Amoco Chemicals
21	6.98	(93)	Tenn Eastman Co
22	6.93	(92)	Dual Lite WFG
23	6.67	(96)	Phillips 65 Company
24	6.50	(92)	Olay Company
25	6.34	(97)	Holnam Inc Dundee Cement

Source: adapted from Airs Data Web site: http://www.epa.gov/airs/

Enclosed on the next page is a copy of EPA's Air Quality Guide. This document describes, from a human health standpoint, the environmental conditions which are related to air quality and what should be done in cases of poor air quality and high ozone levels. Remember, as you read this pamphlet, that the EPA standards are primarily based on human health standards; plants are effected differently than humans. Scientists believe that white pine, for instance, can show adverse effects to ozone exposure when the concentration in the air is as low as 50 ppb over an extended period of time (5-8 hours). Additionally, some humans are also more susceptible at lower, more chronic levels of ozone—this was the primary reason EPA switched to a different ozone standard (80 ppb averaged over an 8-hour period) in July of 1997.

A major objective of both EPA and Forest Watch is to monitor the impacts of this pollutant, ozone, on ourselves and our natural environment. By participating in Forest Watch, students are helping scientists better understand these interactions and to affect a positive change for plant life and for our own living conditions.

EPA Air Quality Guide

Color Coded Index	Weather Conditions	Recommended Action
Unhealthful	■ Hot (middle 90s to 100s), hazy and humid ■ Stagnant air ■ Little change of rain ■ Stationary high pressure system with sunny skies ■ Temperatures in the upper 80s and 90s ■ Light winds	**When air quality reaches unhealthful levels:** ■ Children and elderly individuals should reduce outdoor activities. ■ Healthy individuals should limit strenuous outdoor work or exercise, particularly between 1 pm-7pm. ■ Individuals with heart or respiratory ailments, emphysema, asthma, or chronic bronchities should limit their outdoor activities. If breathing becomes difficult, move indoors. **When air quality is forecast to reach unhealthful levels, citizens are strongly urged to:** ■ Limit driving and, when possible, combine errands. ■ Use area bus and rail lines, or share a ride to work. ■ Avoid mowing lawns with gasoline powered mowers.
Approaching Unhealthful	■ Slow moving high pressure system with sunny skies ■ Mild summer temperatures (upper 70s to mid 80s)	**When air quality approaches unhealthful levels, citizens are urged to:** ■ Avoid mowing lawns with gasoline powered mowers. ■ Carpool and .or take public transportation, when possible. ■ Avoid refueling automobiles on days when levels of smog are predicted to be high.
Moderate	■ Light to moderate winds (15 knows or less) ■ High pressure system with partly cloudy or sunny skies ■ Cool summer temperatures (mid 70s to low 80s)	**When air quality is in the moderate range, citizens should:** ■ Consolidate automobile trips and errands. ■ Limit can and truck idling when possible. ■ Conserve electricity and set air conditioners to 78° F. ■ Refuel cars after dusk.
Good	■ Windy conditions (15-20 knots or higher) ■ Heavy or steady rain ■ Passing cold front carries pollution out of area	**Thoughout the ozone smog season (May through September), citizens should make an extra effort to minimize air pollution emissions;** ■ Follow refueling use instructions for efficiency of vapor recovery systems, and do not top off the tank. ■ Carpool, use mass transit, bike, or walk when possible. ■ Keep cars and boats tuned-up. ■ Use environmentally safe paints and cleaning products. ■ Make sure the car's gas cap fits properly

Air Quality Hotline (800) 821-1237

What You Should Know About Ozone

- Ozone is a major element of urban smog. Ozone can reduce lung function (for example, by limiting the ability to take a deep breath), and can cause coughing, throat irritation and breathing discomfort. There is also evidence that ozone can lower resistance to respiratory disease, such as pneumonia, damage lung tissue and aggravate chronic lung disease, such as asthma or bronchitis.
- Children and those with pre-existing lung problems (such as asthma) are sensitive to the health effects of ozone. Even healthy adults who perform physical exercise or manual labor outdoors can experience the unhealthful effects of ozone.

What is ozone?

Ozone is a colorless gas that can be found in the air we breathe. Each molecule of ozone is composed of three atoms of oxygen, one more than the oxygen molecule which we need to breathe to sustain life. The additional oxygen atom makes ozone extremely reactive. Ozone exists naturally in the earth's upper atmosphere, the stratosphere, where it shields the earth from the sun's ultraviolet rays. However, ozone found close to the earth's surface, called ground-level ozone, is a harmful air pollutant.

Where does ground-level ozone come from?

Ozone is formed by a chemical reaction between volatile organic compounds (VOCs) and oxides of nitrogen in the presence of sunlight. Sources of VOCs and oxides of nitrogen include:
1) automobiles, trucks and buses; 2) large industry and combustion sources such as utilities;
3) small industry such as gasoline dispensing facilities and print shops; 4) consumer products such as paints and cleaners; and 5) off-road engines such as aircraft, locomotives, construction equipment and lawn and garden equipment. Ozone concentrations can reach unhealthy levels when the weather is hot and sunny with relatively light winds.

How does ozone affect human health?

Even at relatively low levels, ozone may cause inflammation and irritation of the respiratory tract, particularly during physical activity. The resulting symptoms can include coughing, throat irritation, and breathing difficulty. Inhaling ozone can affect lung function and worsen asthma attacks. Ozone can increase the susceptibility of the lungs to infections, allergens, and other air pollutants. Medical studies have shown that ozone damages lung tissue and that after exposure has ended complete recovery may take several days.

What are the new ozone standards?

In 1997, EPA strengthened national standards for ground-level ozone. The new standard, set a level of 0.08 ppm averaged over an 8-hour period, is based on the best and most recent health effects and scientific information. As EPA Administrator Carol Browner recently stated, "These new standards will provide health protection to 125 million Americans, including 35 million children."

Who is sensitive to ozone?

Groups that are sensitive to ozone include children who are active outdoors, outdoor workers, and people with respiratory disease, such as asthma. Sensitive people who experience effects at lower ozone concentrations are likely to experience more serious effects at higher concentrations.

Air Quality	Pollutant Standards Index (PSI) PSI=100 corresponds to EPA's air quality Standard	Ozone Concentration (parts per million) 8-hour average unless noted
Good	0 to 50	0.0 to 0.064
Moderate	51 to 100	0.065 to 0.084
Unhealthy for Sensitive Groups	101 to 150	0.085 to 0.104
General Unhealthy	151 to 200	0.105 to 0.124
Very Unhealthy	200 to 300	0.125 (8-hr) to 0.404 (1-hr)

For More Information Visit EPA's Web site at:

www.epa.gov/airnow

United States Environmental Protection Agency

Office of Air & Radiation

FACT SHEET

HEALTH AND ENVIRONMENTAL EFFECTS OF GROUND-LEVEL OZONE

Why are We Concerned about Ground-Level Ozone?

- Ozone is the prime ingredient of smog in our cities and other areas of the country. Though it occurs naturally in the stratosphere to provide a protective layer high above the earth, at ground-level it is the prime ingredient of smog.
- When inhaled, even at very low levels, ozone can:
  - cause acute respiratory problems;
  - aggravate asthma;
  - cause significant temporary decreases in lung capacity of 15 to over 20 percent in some healthy adults;
  - cause inflammation of lung tissue;
  - lead to hospital admissions and emergency room visits [10 to 20 percent of all summertime respiratory-related hospital visits in the northeastern U.S. are associated with ozone pollution]; and
  - impair the body's immune system defenses, making people more susceptible to respiratory illnesses, including bronchitis and pneumonia.

Who is Most at Risk from Exposure to Ground-Level Ozone?

- Children are most at risk from exposure to ozone:
  - The average adult breathes 13,000 liters of air per day. Children breathe even more air per pound of body weight than adults.
  - Because children's respiratory systems are still developing, they are more susceptible than adults to environmental threats.
  - Ground-level ozone is a summertime problem. Children are outside playing and exercising during the summer months at summer camps, playgrounds, neighborhood parks and in backyards.
- Asthmatics and Asthmatic Children:
  - Asthma is a growing threat to children and adults. Children make up 25 percent of the population and comprise 40 percent of the asthma cases.
  - Fourteen Americans die every day from asthma, a rate three times greater than just 20 years ago. African-Americans die at a rate six times that of Caucasians.
  - For asthmatics having an attack, the pathways of the lungs become so narrow that breathing becomes akin to sucking a thick milk shake through a straw.
  - Ozone can aggravate asthma, causing more asthma attacks, increased use of medication, more medical treatment and more visits to hospital emergency clinics.
- Healthy Adults:
  - Even moderately exercising healthy adults can experience 15 to over 20 percent reductions in lung function from exposure to low levels of ozone over several hours.
  - Damage to lung tissue may be caused by repeated exposures to ozone — something like repeated sunburns of the lungs — and this could result in a reduced quality of life as people age. Results of animal studies indicate that repeated exposure to high levels of ozone for several months or more can produce permanent structural damage in the lungs.
  - Among those most at risk to ozone are people who are outdoors and moderately exercising during the summer months. This includes construction workers and other outdoor workers.

How does Ground-Level Ozone Harm the Environment?

- Ground-level ozone interferes with the ability of plants to produce and store food, so that growth, reproduction and overall plant health are compromised.
- By weakening sensitive vegetation, ozone makes plants more susceptible to disease, pests, and environmental stresses.
- Ground-level ozone has been shown to reduce agricultural yields for many economically important crops (e.g., soybeans, kidney beans, wheat, cotton).
- The effects of ground-level ozone on long-lived species such as trees are believed to add up over many years so that whole forests or ecosystems can be affected. For example, ozone can adversely impact ecological functions such as water movement, mineral nutrient cycling, and habitats for various animal and plant species.
- Ground-level ozone can kill or damage leaves so that they fall off the plants too soon or become spotted or brown. These effects can significantly decrease the natural beauty of an area, such as in national parks and recreation areas.
- One of the key components of ozone, nitrogen oxides, contributes to fish kills and algae blooms in sensitive waterways, such as the Chesapeake Bay

What Improvement Would Result from EPA's New Standards?

EPA's new ozone standards will provide increased protection beyond that provided by the previous standard from the following effects:

- Reduced risk of significant decreases (15% to over 20%) in children's lung functions (such as difficulty in breathing or shortness of breath), approximately 1 million fewer incidences each year, which can limit a healthy child's activities or result in increased medication use, or medical treatment, for children with asthma;
- Reduced risk of moderate to severe respiratory symptoms in children, hundreds of thousands of fewer incidences each year of symptoms such as aggravated coughing and difficult or painful breathing;
- Reduced risk of hospital admissions and emergency room visits for respiratory causes, thousands fewer admissions and visits for individuals with asthma;
- Reduced risks of more frequent childhood illnesses and more subtle effects such as repeated inflammation of the lung, impairment of the lung's natural defense mechanisms, increased susceptibility to respiratory infection, and irreversible changes in lung structure. Such risks can lead to chronic respiratory illnesses such as emphysema and chronic bronchitis later in life and/or premature aging of the lungs; and
- Reduce the yield loss of major agricultural crops, such as soybeans and wheat, and commercial forests by almost $500,000,000.

Background: What is Ground-level Ozone?

- Ozone is not emitted directly into the air, but is formed by gases called nitrogen oxides (NOx) and volatile organic compounds (VOCs) that in the presence of heat and sunlight react to form ozone. Ground-level ozone forms readily in the atmosphere, usually during hot weather.
- NOx is emitted from motor vehicles, power plants and other sources of combustion. VOCs are emitted from a variety of sources, including motor vehicles, chemical plants, refineries, factories, consumer and commercial products, and other industrial sources.

Changing weather patterns contribute to yearly differences in ozone concentrations from city to city. Also, ozone and the pollutants that cause ozone can be carried to an area from pollution sources located hundreds of miles upwind.

STUDY SITE PROTOCOLS

C

CONTENTS

ESTABLISHING THE PIXEL-SIZED SAMPLING PLOT (PSSP)

BACKGROUND

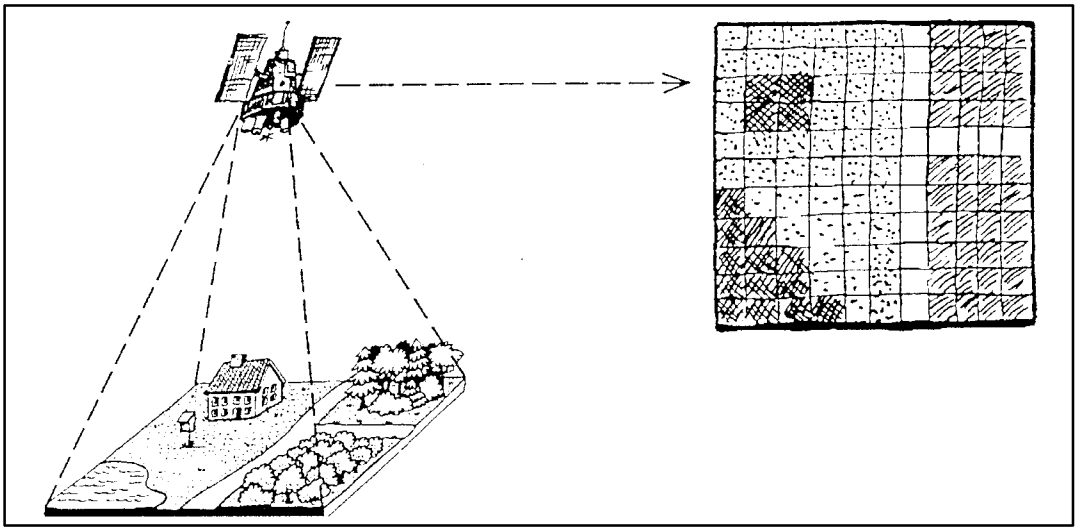
Guiding Question

What is an appropriate size for our sampling plot?

To conduct our long-term monitoring study, we will select a permanent location that contains the five permanently tagged white pine trees from which you and future students will sample each year. The plot size is designed to match the 30 m x 30 m pixel size of the Landsat Thematic Mapper (TM) satellite image. The word pixel stands for "picture element." Pixels are the individual colored squares that make up a satellite computer image.

Because the sampling plot is the size of a Landsat TM pixel, it is called a pixel-sized sampling plot (PSSP).

In this activity, you will establish a permanent 30 m x 30 m PSSP with a north-south orientation to match the Landsat TM north-south (polar) orbit. You will use a compass and either a field measuring tape or a pacing method to measure the length of the sides of the PSSP.



This represents how a satellite views the earth's land cover as a group of equal size units placed on a landscape. Each unit is called a pixel. Jan 'Smolik 1996 TEREZA Association for Environmental Education, Czech Republic

Discussion Questions

1. Why is the size of the PSSP 30 m x 30 m?
2. Why do we need a permanent study plot?
3. Why is the PSSP oriented north-south?
4. What is the length of your pace?
5. How do you measure a 30-m side using the pacing method?

ESTABLISHING THE PIXEL-SIZED SAMPLING PLOT (PSSP)

PROTOCOL

How can you measure and establish your PSSP?

Materials

- 50-meter field tape (OR measure in English and convert to metric)
- magnetic compass
- plot corner markers – stakes, coat hangers or thin wooden dowels and flagging tape

Procedure

You will work with at least one partner for this activity.

1. Within your forest stand, place a marker at a location designated by your instructor.
2. Establish side 1 of your PSSP**.** Your instructor will tell you to measure a length 30 meters long toward either the north or south of your initial marker. Place a 2nd marker at the end of this 30-meter side.
3. Establish side 2 of your PSSP. From the 2nd marker, measure another 30-meter side perpendicular to side 1. Place a 3rd marker at the end of this 30-meter side.
4. Establish side 3 of your PSSP. From the 3rd marker, measure another 30-meter side perpendicular to side 2. Place a 4th marker at the end of this 30-meter side.
5. Establish side 4 of your PSSP. From the 4th marker measure another 30-meter side perpendicular to side 3. You will have been successful if your final 30-meter side ends at or within 1 meter of your 1st marker. If your final measurement is more than one meter from the first marker, redo steps 2-5, being careful to measure the 30-m lengths and use the compass correctly.

   If you are far from the mark, check compass headings for each side again. Make sure they are either north-south orientations or east-west orientations. Check the length of each side making sure they are each 30 meters long.

Analysis and Interpretation

1. How close did your final leg come to your first corner marker?

2. What might be some reasons for not hitting the first corner marker?

3. What other ways might you lay out the PSSP?

4. What were some of the problems you encountered while choosing or measuring your PSSP?

5. What skills have you learned and how might you use them in other situations?

6. Why do we use a 30 m x 30 m PSSP?

PIXEL-SIZED SAMPLING PLOT (PSSP)

TEACHER RESOURCE

Introduction

In this protocol, students will establish a Pixel-Sized Sampling Plot (PSSP). The plot, a 30 m x 30 m square, is based on Landsat Thematic Mapper (TM) images which use a 30 m^2 pixel (see the Remote Sensing Primer for detailed background information). The five white pine trees from which you will collect your samples each year should be located within the PSSP.

Guiding Question

What is an appropriate size for our sampling plot?

Materials

- 50-meter field tape (OR measure in English and convert to metric)
- magnetic compass
- plot corner markers – stakes, coat hangers or thin wooden dowels and flagging tape (these may be used for sites that are densely covered)

Background

The purpose of the PSSP is to establish a permanent location from which white pinesamples are taken each year. Within the PSSP, understory/ground cover and canopy closure measurements may be used in conjunction with satellite data. Ultimately, both types of information may help us assess the health of New England forests. Students will use compasses and field measuring tapes (see Management Suggestions for alternative pace method) to establish the perimeter of the plot on a north-south orientation. Diagonals across the plot will later be established for taking measurements of canopy closure and ground cover. Details can be found in the Understory/Ground Cover and Canopy Closure Protocol.

The location of the PSSP should be within a forested stand. Ideally, the PSSP would be found in a white pine forest stand. However, this forest composition may not be readily available to you and your site may consist of a combination of white pine and other species, for example, in a mixed coniferous/deciduous forest.

The 30 m x 30 m sampling plot size is used because is matches the TM pixel size, it is usually large enough to contain at least five white pine trees and it will allow students to see how big a pixel is and how much vegetation it may contain.

Management Suggestions

The PSSP should be located within a few hundred meters of the school. This will allow for greater accessibility on a regular basis. If the selected location is not on school property, you may need to seek permission of the land owner. If your site is disturbed in such a way that it interferes with permanent sampling, a new location should be established.

The PSSP can be selected during either the spring or the fall. Ground conditions should allow for easy access for students. A quick survey of your site will help you to decide on a general location for your plot, which should be located away from the edge of a forest stand, road or major trail which could impact the health of the trees nearby. Try to select a location for the PSSP that is level and devoid of briars, poison ivy, dense undergrowth, etc. It will be important that you visit several potential sites, if possible, and select the best one for student activities. Ideally, the PSSP will contain mature white pine trees that reach the forest canopy and that offer access to the middle third of their functional canopy. Trees that are too tall need to be avoided, as do young understory trees.

Because you will sample from the same PSSP each year, it may be beneficial to permanently mark at least one corner. A re-bar stake set nearly flush with ground level will be easy to locate each year. Another option may be to use a landscape feature, such as a large tree or rock, a known distance from one corner of your plot. Establish the placement of the first marker so that the entire PSSP will be within the forest. If possible, avoid stands that are smaller than the final PSSP.

Students may be assigned to work in teams for setting up the PSSP and its diagonals**.**

You may want students to determine their pace length before laying out the PSSP. Pacing may be used to establish the perimeter lengths and diagonals instead of using a field tape measure. See Additional Suggestions below for measuring pace.

Students may not be familiar with transects. Transects are established lines along which samples or measurements are taken at particular intervals. In this instance, transect is synonymous with the PSSP diagonals to be used in the Understory/Ground Cover and Canopy Closure Protocol.

The PSSP may be established in any number of ways as long as it is on a north - south orientation, but make sure you design it so the final plot contains your five chosen trees! (See Tree Selection and Tagging Protocol for details.) One objective of the student protocol is to have students learn to use various field techniques. These involve the use of compasses, sighting, pacing to measure distances and using field tape measures. Students may also learn to use the Pythagorean Theorem to establish the lengths of the diagonals in the Understory/Ground Cover and Canopy Closure Protocol.

Sighting on an object at a distance is very important when using a compass. A student using a compass should keep the student who is measuring a perimeter side on course. While sighting on a distant object, the compass user instructs the student who is measuring to move to the left or right accordingly. This communication helps to keep the sides directly on a north-south or east-west orientation.

After the students have established the perimeter of the PSSP, lead a brief discussion of the process (see the Questions & Answer Key for hints in leading this discussion). Students may list several responses for question #2 such as problems with using the compass and sighting, pacing inaccurately to measure the distance and/or obstacles such as trees within their line of sight.

Additional Suggestions

Measuring Pace Length:

This activity can be done inside in a hallway or some other convenient location.

Rough estimates of transect length or the size of a square or rectangular study plot are often used in fieldwork. A simple way of doing this type of estimation is to pace out the desired distances. By knowing the length of one's pace, and by counting the number of paces needed to cover the distance between two spots, a close estimate of the distance can be calculated.

A pace equals 2 steps: one with the right foot and then one with the left foot (or vice versa). Students should use a normal stride length when pacing. Lay either a 20- or 50-m field tape on the floor. Students should start at the zero mark on the tape by placing their heels at this mark. Students then walk the required distance and count the number of steps they take. Round to the nearest step at the end. To maximize accuracy in determining pace, use a long tape measure and perform repeated trials and average.

Sample data and calculations:

Trial #1 39 steps

Trial #2 41 steps

Trial #3 40 steps

Average number of steps = 40 steps

Average number of paces = 40 steps/ 2 steps per pace = 20 paces

Distance paced = 30 meters (known length using a tape measure)

Pace length = 30 m / 20 paces = 1.5 m/pace

If the student who measured the above pace length needs to pace a 30 m perimeter side, then he/she will simply divide the side length by his/her pace length (1.5 m/pace). Ex: 30 m / 1.5 m/pace = 20 paces.

This student would measure the perimeter leg by walking 20 paces.

Using compasses and pacing:

Have your students practice another activity using compasses and pacing. A mini-orienteering course located in your forest stand helps students develop these skills.

See **Be Expert With Map & Compass - The Orienteering Handbook** by Bjorn Kjellstrom.

Answer Key

1. How close did your final leg come to your first corner marker?

   Answers will vary.

2. What might be some reasons for not hitting the first corner marker?

   Answers should relate to problems with sighting and use of a compass, pacing distance, trees or terrain interfering with movement along sight lines, etc.

3. What other ways might you lay out the PSSP?

   Surveyors transit, start at the center and lay out the diagonals first with end points being the NE-NW-SE-SW corners, etc.

4. What were some of the problems you encountered while choosing or measuring your PSSP?

   Finding a suitable PSSP location which included five usable white pines, or any of the answers found in question 2.

5. What skills have you learned and how might you use them in other situations?

   Compass skills in outdoor activities, orienteering, laying out house or garden plots, etc.

6. Why do we use a 30 m x 30 m PSSP?

   It coincides with the sizes of an individual pixel of a Landsat TM satellite image (see Remote Sensing section for further information).

UNDERSTORY/GROUND COVER AND CANOPY CLOSURE

BACKGROUND

Guiding Questions

How do forests change over time?

How can we measure the change?

What contributes to this change?

What are important elements of our PSSP?

The goal of site assessment is to acquire a general awareness of the forest and plant community in your PSSP and to observe how environmental factors may influence that community. For this protocol, you will use a visual assessment of vegetation to determine the extent of both overhead and forest floor cover of the PSSP. You will observe the forest canopy by using a tool called a densiometer. You can use the information from the densiometer to calculate the percent canopy cover.

Understory/ground cover, in this study, is defined as any vegetation that is smaller in diameter than 10 cm (about 4 in.). Understory/ground cover measurements are important because they may be an indication of the amount of sunlight that is reaching the forest floor. If the canopy is dense, then it may be blocking the sunlight from reaching the forest floor. This will obviously effect the vegetation that is growing there! In addition, the extent of the canopy has a direct influence on the measurements made by a satellite that will be used to determine the amount of vegetation present in an area.

In this protocol, you may also use a vegetation guide to identify some of the common shrubs in the plot such as blueberry, elderberry, honeysuckle or sweet fern. Common groundcover (generally herbaceous vegetation) includes grasses, ferns and wildflowers. Some of the low vegetation is called the understory and includes the tree saplings and taller bushes growing underneath the canopy.

Discussion Questions

1. What are canopy closure and understory/ground cover?

2. How are canopy closure and understory/ground cover related?

3. How might this relationship change over time (seasonally, yearly, etc)?

UNDERSTORY/GROUND COVER AND CANOPY CLOSURE

PROTOCOL

How can you measure canopy closure and understory/ground cover?

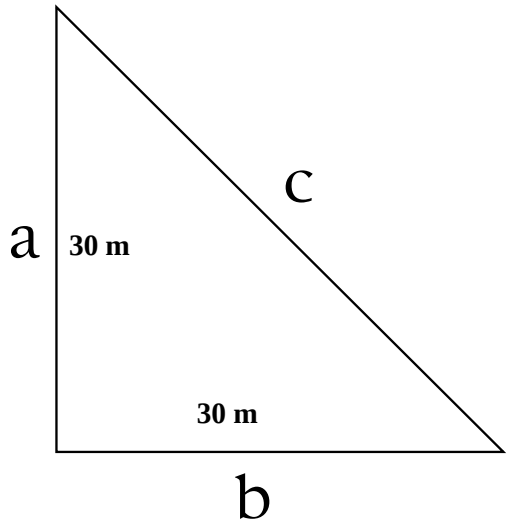
How can you use information about the canopy closure and understory/ground cover to assess change in the forest?

Materials

- handmade densiometer
- tree, wildflower, shrub identification guide books
- Understory and Ground Cover and Canopy Closure Data Sheet

Determining the diagonals of the PSSP:

To calculate the expected length of your PSSP diagonals you can use the Pythagorean Theorem, where a, b, & c are three sides of a right triangle.



Example:

$a^2 + b^2 = c^2$

(_____m)^2+ (_____m)^2 = c^2

_____m^2 +_____ m^2 = c^2

_____ m^2 = c^2

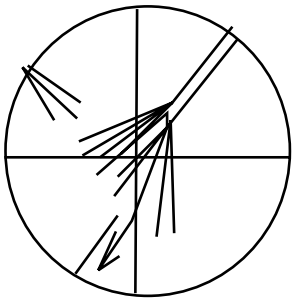
$\sqrt{\text{_____}}$ m^2 = c^2

Length diagonal (c) =_____ m

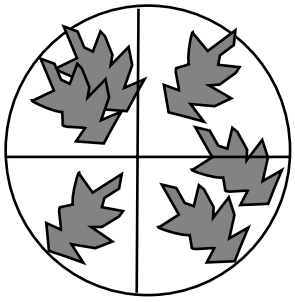
1. Work with one or two other members of your team. Station team members at opposite corners of the PSSP. Have a third member pace the diagonal between them. If there are too many trees and shrubs in the way, you may need to use a compass to establish the diagonal using the steps below:
   a. Obtain a compass and stand at one corner of your PSSP, sight along the direction of one of its established sides (along a north-south or east-west orientation).
   b. Now, read your compass bearing. Add 45° to that number. Turn to face that new compass heading.
   c. You should now be looking towards the opposite corner of the PSSP along the plot diagonal.
   d. You may want to place a few markers along each diagonal to establish a straight line to the opposite corner. You should follow this line during this activity.

Understory/Ground Cover and Canopy Closure Measurement

2. Once you have established the PSSP diagonals, you may begin the data collection. Start at any corner of the PSSP. Begin by walking along the diagonal toward the opposite corner of your plot. You will be stopping to gather data about every pace length (2 steps) along the way across. Depending upon your actual pace length, you will probably make about 20 observations as you traverse each diagonal of the PSSP.
3. At each observation point (each pace) stop and look up at the canopy through your handmade densiometer. As you look up through the bottom of the tube, make certain that the metal weight (nut or washer) is hanging directly below the intersection of the two strings that make up the crosshair at the top of the tube. This will ensure that you are looking straight above you, rather than off at an angle.
4. Focus on the crosshair. If you can see branches, needles or leaves at the <u>intersection</u> of the crosshair, your partner should record a (+) on the data sheet. If there is only sky seen at the crosshair, your partner should record a (-). Continue to make measurements until you reach the opposite corner of the PSSP. Don't worry if you use fewer recording spaces than appear on the data sheet. You should, however, expect to have approximately 20 data points.



Record a (+), branches and needles seen at cross hairs



Record a (-), branches and needles seen at cross hair, but rather sky is seen

5. Each time you make a canopy closure observation, you will also make a understory/ground cover observation. You won't need the densiometer for this observation. Remain standing in the same spot as you were when you looked up at the canopy, but now look down around your feet.
6. If you observe <u>any</u> live vegetation including herbs, bushes, shrubs and small trees with a diameter less than 10 cm (about 4 in.) at the spot you are standing on, tell your partner to mark a (+) on the data sheet. If not, for example if you are standing in dead leaf litter or bare ground, mark a (-) on your data sheet.

7. Repeat the canopy closure and understory/ground cover observations and data collection along the opposite diagonal of the PSSP.
8. Calculate the percent canopy closure and the percent understory/ground cover. These are two separate calculations, don't combine the data! Calculate the percentage for each category as follows:

(Total +'s / Number of observation along both diagonals) x 100 = % closure or cover

For Example:

Canopy Closure: (20 "+" / 40 observations) x 100 = 0.50 x 100 = 50% canopy closure

Understory/ground cover: (30 "+" / 40 observations) x 100 = 0.75 x 100 =
75% Understory/ground cover

UNDERSTORY/GROUND COVER AND CANOPY CLOSURE DATA SHEET

Students/Class:_____ Date: _____

School:_____ Teacher:_____ Town:_____

Tree #'s:_____ Site Coordinates (long. and lat.):_____

1. Describe the natural terrain and human features associated with your PSSP:

2. **Canopy Closure Data:** If you are asked to do canopy closure/composition observations, instead of using a "+" use "c" for coniferous trees, "d" for deciduous trees, or "cd" if both are present.

   Diagonal #1: ___

   Diagonal #2: ___

   Calculate the Percentage Of Canopy Closure: ("+" /total number of measurements) x 100 = % canopy closure. (show calculations)

   Percent Canopy Closure: _____ %

   Would you describe your canopy as Closed (almost complete), Moderate, or Limited (low leaf coverage). Circle one. What was the percent canopy closure measured last year?

3. **Ground/Understory Cover Data:**

   Diagonal #1: ___

   Diagonal #2: ___

   Calculate the Percentage Of Understory/Groundcover Cover: (# of "+" / total number of measurements) x 100 = % groundcover. (show calculations)

   Percent Understory/Groundcover Cover: _____ %

   Would you describe your ground/understory cover as Total, Moderate, or Limited (low plant coverage)? Circle one. What was the percent understory/ground cover measured last year?

Average your data with others in your class and transfer the information to the Master Data Sheet.

Analysis and Interpretation

1. What resources do plants compete for in your study site?

2. Which tree species is most dominant in your PSSP? Is it the white pine or some combination of white pine and other tree species? How might canopy closure by the trees in your PSSP affect shrubs and understory/ground cover beneath?

3. If available, compare data from previous years to the data you have just collected. Has there been a change over time in the canopy closure and understory/ground cover?

4. How can you use information about the canopy closure and understory/ground cover to assess change in a forest over time?

5. How do these assessments possibly relate to forest health?

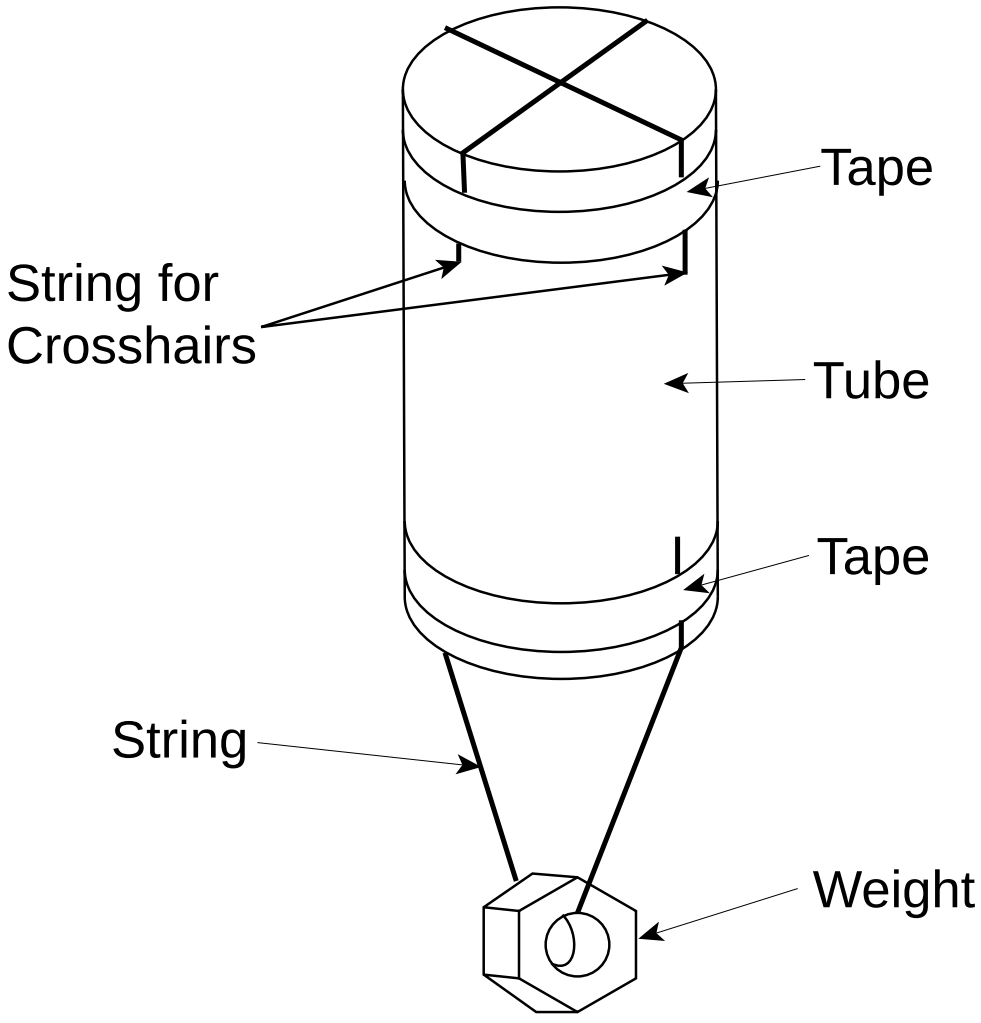
DENSIOMETER CONSTRUCTION

Materials

- tube - approximately 11.5 cm (4.5 in.) long and 4 cm (1.5 in.) in diameter
- string
- metal washer or nut (make sure this weight is heavy enough to hang easily)
- tape

Procedure

1. Attach two pieces of string (each about 10 cm long) across the opening at one end of the tube.
2. Attach another piece of string (about 20 cm long) to both sides of the other end of the tube with the washer/nut hanging about 7.5 cm (3 in) down from the tube.



UNDERSTORY/GROUND COVER AND CANOPY CLOSURE

TEACHER RESOURCE

Introduction

This activity will help students become aware of some of the characteristics of the site in which their trees are located. Students will examine canopy and sapling/shrub/ground cover. Over long periods of time, changes in the relative percentages of these factors may help show how the plant communities are changing, for example, through stages of succession. An immediate application of this information is that it can be used as a "ground truth" tool for the interpretation of satellite images. It also helps us to describe the study site so that comparisons can be made between schools and study sites. Assessment of the PSSP within your study site should occur while the deciduous trees are fully leafed-out.

Guiding Questions

How do forests change over time?

How can we measure the change?

What contributes to this change?

What are important elements of our PSSP?

Materials

- Understory/Ground Cover and Canopy Closure Data Sheet
- handmade densiometer
- tree, wildflower, shrub identification guide books
- magnetic compass and flagging tape may be needed for canopy closure observations

Background

The goal of site assessment is to acquire a general awareness of the forest and plant community in your PSSP and to observe how environmental factors influence that community. For this protocol, you will use a visual assessment of vegetation to determine the extent of both overhead and forest understory cover of the PSSP. You will observe the forest canopy by using a tool called a densiometer. You can use the information from the densiometer to calculate the percent canopy cover.

Ground/understory cover, in this study, is defined as any vegetation that is smaller in diameter than 10 cm (about 4 in.). Understory/ground cover measurements are important because they may be an indication of the amount of sunlight that is reaching the forest floor. If the canopy is dense, then it may be blocking the sunlight from reaching the forest floor. This will obviously effect the vegetation that is growing there! In addition, the extent of the canopy has a direct influence on the measurements made by a satellite that will be used to determine the amount of vegetation present in an area.

Students may use a guide to identify some of the common shrubs in the plot such as blueberry, elderberry, honeysuckle, or sweet fern, as well as some common groundcover species (generally herbaceous vegetation) including grasses, ferns and wildflowers. Some of the low vegetation is called the understory and includes the tree saplings and taller bushes growing underneath the canopy. You may want to have the students identify the other trees making up the canopy such as oak, maple, birch, or beech.

Management Suggestions

Method and Procedure

You should need no more than half a class period to make the densiometers. The directions for making this instrument are based on using a toilet paper tube but PVC pipe is another, more permanent, option.

It is highly recommended that you use a composition notebook to keep a log of all observations made in the PSSP. You can assign this task to one of your reliable students.

Depending on how far you have to walk to get to your PSSP, site assessment may take more than one class period. Divide the class into teams to complete the site assessment data sheet. Stagger starting times along the diagonals so groups are not tripping over one another.

Calculate the percentage for **each** category (canopy closure and understory/ground cover) as follows:

(Total +'s / total number of measurements) x 100 = %(canopy closure or understory/ground cover)

For Example:

Canopy Closure: (20 "+" / 40 observations) x 100 = 0.50 x 100 = 50%

Understory/Ground Cover: (30 "+" / 40 observations) x 100 = 0.75 x 100 = 75%

Important note: In the field, obstacles such as trees or boulders may interfere with observations. If so, try the following suggestions:

A. If an obstacle such as a tall tree lies on the diagonal, step to either side of the obstacle and make your observation or make the observation just before or beyond the obstacle.
B. If your plot is heavily vegetated you may need to place marker flags like those used to mark the corners of your PSSP along the diagonals. Remember to collect all markers from the field after you have finished your canopy closure and understory/ground cover measurements.

An alternative to using a "+" to indicate the presence of canopy cover is to be more specific and use a "c" for a coniferous tree, a "d" for a deciduous tree, or "c/d" if both types are present. Whichever symbol you decide on, stress the importance of indicating the presence of canopy only if branches, needles or leaves are seen at the crosshair. If needles or leaves are visible through the densiometer tube but sky is seen at the crosshair, a "-" should be recorded.

Discuss with students the meanings of shrub, tree sapling and groundcover vegetation. Students should collect data only on the live vegetation. A "+" indicates that a shrub, sapling (< or = 10 cm in diameter or to 4 in.) or groundcover is at the spot where he/she stops at a particular point along the diagonal. A "-" means the absence of any living vegetation touching the student.

Evaluation

Make sure several teams repeat the observations for each diagonal. Have students compare their data as a form of quality control. Brainstorm reasons why the data may not all be the exactly the same. Decide if you will report your data as an average of all the data for each category. Are there some observations that are vastly different from the others? Should these be included in the overall report?

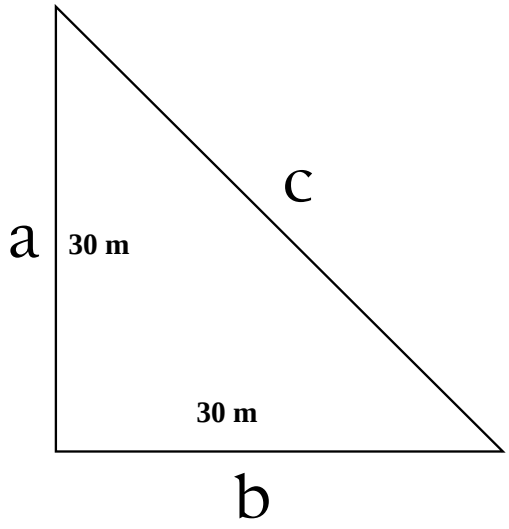
Additional Suggestions

On the surface it would seem reasonable that there should be a direct, inverse relationship between percent canopy closure and percent understory/ground cover. If there is 80% canopy closure, there should be about 20% understory/ground cover. The following demonstration can illustrate to students why this probably is not so. Draw a circle with a 10 cm radius on a sheet of paper. Make random holes through the circle so that about 80% of the original circle's total area is undisturbed. Darken the classroom, hold the sheet of paper above a table, and shine a light (the light mimics the sun) over the large circle which represents the forest canopy. Students should be able to see, as you slowly move the position of the light, that there is light filtering through "gaps" in the canopy and that, as the sun travels overhead during the course of a day, a plant will receive light directly through several gaps.

Understory plants and shrubs have adaptations allowing them to flourish under these varying conditions. Students may compare vegetation to see if there are differences between plants of the same species growing in a field and in the forest understory. Some groundcover species emerge and flower very early in the spring before the canopy leaves are out.

Answer Key

Determining the diagonals of the PSSP:



Example:

	a^2 +	b^2	=	c^2
$(\underline{30}\ m)^2$	$+(\underline{30}\ m)^2$		=	c^2
$\underline{900}\ m^2$	$+\underline{900}\ m^2$		=	c^2
	$\underline{1800}\ m^2$		=	c^2
$\sqrt{}$	$\underline{1800}\ m^2$		=	c^2
Length diagonal (c)			=	$\underline{42.4}$ m

Analysis and Interpretation

1. What resources do plants compete for in your study site?

   Plants compete for light, water, soil nutrients and space.

2. Which tree species is most dominant in your PSSP? Is it the white pine or some combination of white pine and other tree species? How might canopy closure by the trees in your PSSP affect shrubs and understory/ground cover beneath?

   White pine is one of the first, if not the first tree, to germinate and recolonize a clear-cut or fire damaged area. In the northeast, it grows quickly in sandy, well-drained soils along the coast and river valleys, becoming a dominant member of the forest community. In white pine stands, it forms a year-round, nearly complete canopy that effectively blocks almost all sunlight from reaching the forest floor, virtually eliminating most groundcover. It is also found scattered throughout mixed hardwood forests of oak/hickory and beech/maple as it is quick to exploit an open area in the canopy. In these areas the canopy is more open during the early spring and late fall as the deciduous trees are leafless. This allows groundcover plants to temporarily exploit open areas of the forest floor.

3. If available, compare data from previous years to the data you have just collected. Has there been a change over time in the canopy closure and understory/ground cover?

   If the data is available, an increase in canopy cover may show a decrease in understory/ground cover and vice versa. In general, there is an inverse relationship between canopy cover and groundcover. The type of ground/understory cover could change over time, though, too. For example, if a more shade tolerant set of species replaces the shade intolerant species, the percent cover could remain the same or even increase!

4. How can you use information about the canopy closure and understory/ground cover to assess change in a forest over time?

   Natural succession in forests containing white pine lead to climax communities composed primarily of hardwoods. This pattern of growth may take hundreds of years to complete. Over the short term, in mixed white pine/deciduous forests, a decrease in the percentage of canopy cover might be an indicator of a decrease in forest health, with trees losing a significant proportion of the actively growing canopy. This loss may be natural or human induced (i.e. pollution events).

QUALITATIVE SITE ASSESSMENT

BACKGROUND

What kinds of observations can you make about your entire PSSP?

Everyday you practice the skill of observation. In fact, you are probably very good at it. But how often have you realized that when you were making observations they were helping to form your judgments and actions? In this protocol, you will make observations at a basic, information-gathering level that will help you better characterize your study area.

Observations are an essential part of any scientific investigation and there are many different types that you can make. Some involve direct measurements, and some are descriptive and more general in quality. So, in addition to some very specific measurements that you will be making about a few of the trees in your PSSP, you will also want to know what else is going on in and around your study area.

One observation in this protocol will involve identifying as many understory species as possible using a vegetation identification booklet and individual experience. While this is not a direct measurement, it is still an important recorded observation that helps describe the site. Another type of observation involves taking pictures of your PSSP and study trees in order to establish a permanent visual record.

Eventually, the observations that you make in this protocol may come in handy if new questions come up about your forest site. And they could help in the interpretation of the measurement results you will obtain in other protocols. In addition, these descriptions are important when we compare study areas between different schools.

It is imperative that you begin to think of the forest as a system and not just a place where independent, unconnected processes are going on. It is difficult for anyone to get a whole-system perspective when only making very detailed measurements of a few components of the PSSP. These broader observations, therefore, should help provide a greater view. So stand back and make some bigger picture, more generalized observations.

QUALITATIVE SITE ASSESSMENT

PROTOCOL

Materials

- camera and film
- vegetation field guides
- PSSP Qualitative Site Assessment Data Sheet

Procedure

1. Record the current weather conditions by circling the appropriate choices on the data sheet.
2. Obtain a camera and film and take some pictures of your PSSP. Take pictures of the ground, the canopy and the surrounding forest. You could start your photographing by taking pictures from each corner of the PSSP while facing in towards the center. After each shot, record on a separate list, the picture number of the film, the picture subject, location and school/teacher name. When the film is developed, transfer this descriptive information appropriately to the back of each picture.
3. Take pictures of each of your five tagged trees. Try to get a full shot, if possible, or just take a shot of the canopy. For each picture, record the tree number, your PSSP location and your school/teacher name on a separate list to be transferred to the developed pictures later.
4. Send duplicates of your developed and well-labeled photos to UNH.
5. Use a local vegetation identification key to try to identify as much of the understory vegetation as possible. Record on the data sheet as much detail as you can. For example, if there are five small oak trees in your PSSP, you could record the species name, the number of trees and an estimate of their heights. Instead of counting all the individuals of a very common species, you could record the species name and a percent estimate of its cover. Perhaps, for example, 25% of your PSSP is covered by a fern species. Try to identify the species name and record its percent cover on the data sheet. You might not have time to count all of the individuals of a species such as blueberry, but you could record the fact that blueberry is present and covers about 35% of the forest floor in the PSSP. If you are having trouble identifying a species name, ask your teacher if you may collect a sample to take back to the lab to identify later. Or, draw a picture of the plant and make lots of detailed descriptions for later identification.

   Always make sure that you have permission to collect any plant material from your study site and that you walk with care, trying not to trample any vegetation!
6. Answer all the questions on the data sheet as completely as you can. Jot down any notes that you might want to look up later or ask your teacher about. Remember that any observation could come in handy later on to help you characterize your specific study site.

PSSP QUALITATIVE SITE ASSESSMENT DATA SHEET

Date:

Students' Names:

_____

_____

_____

_____

Teacher/School Name:

_____

PSSP Location:

_____

1.Weather Observations & Notes:

Circle all that apply:

1) Sunny Few Clouds Partly Cloudy Mostly Cloudy
2) Clear Raining Snowing Overcast
3) Cold Warm Hot
4) Windy Buggy Humid
5) Notes on weather conditions:

2. Take Pictures of Site and Tagged Sample Trees (if feasible)

   Note: These pictures are very desirable to help UNH understand your site. Label each picture when developed.

3. Using a vegetation key and/or experience, identify understory vegetation. Include scientific name and common name if you know them. Count or estimate the % cover for each vegetation type, if possible. (Use back of sheet if necessary)

vegetation type	#/% cover	vegetation type	#/% cover
1) _____		6) _____	
2) _____		7) _____	
3) _____		8) _____	
4) _____		9) _____	
5) _____		10) _____	

4. Forest Floor Litter: what makes-up the forest floor? Are there dead leaves, living vegetation or some mixture of the two? Are there dead & down logs? Are lichens or moss evident on the trees, dead logs, or on the forest floor? Record other observations about the forest floor.

5. Describe the topography of the PSSP. Is there a gentle slope to the terrain, is it flat or are you on a hill side? Is it a high site or a low-land site? Are the soils sandy and typically dry or is it moist, containing much top soil?

6. Describe the general location of your PSSP. Are you near a road, a river or stream, or a trail? If yes, how big are these features (i.e., is it a four-lane highway or an infrequently used dirt road)? Are you near any industrial areas? What is the population of the town/city your site is located in? Are there any other observations about the surrounding area that you want to note?

7. Provide a subjective overview of your site and its condition. Is this a purely conifer stand, a mixed stand dominated by white pine, or a stand dominated by another species? Is this a truly forested stand or is it a small island of trees between the soccer field and the school building?

   How would you assess the health of the site just by looking at it from a non-scientific standpoint? Is there evidence of past human use of the land (i.e., Was this an old farm field which has now become overgrown due to disuse?)

8. Describe any evidence or sighting of animals.

9. Are there dead trees? Count them, if possible. Are they recently dead, that is, do they still have green foliage and branches; or have they been long-dead and missing bark? Is there any evidence of animals using the dead trees? Describe what you see.

10. Record any other observations you can make about your study site. Include a drawing or map of your site if you have time.

TREE FORM AND DIAGNOSIS

BACKGROUND

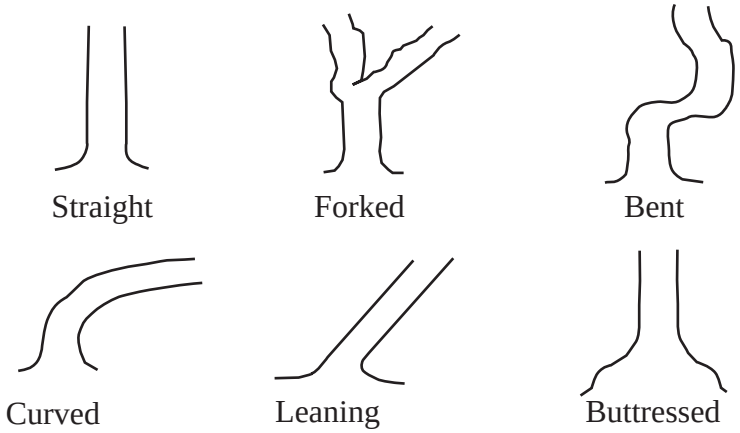
Guiding Question

What is tree form and how can it help us learn about tree health?

Tree form observations describe the shape of a tree and whether or not it is typical of a healthy example of that species. Tree diagnosis looks closely at particular problems that may be affecting a tree's health and its ability to function. Together, tree form and tree diagnosis allows us to assess the general conditions of the forest through our understanding of a smaller part made up of trees.

Every tree species has a characteristic growth pattern and shape. Tree form describes the physical appearance and make-up of the tree. These are called morphological characteristics. Morphological characteristics include crown shape, branching pattern, and the growth of the major stem: forked, bent, arched, buttressed, or straight. These growth patterns may be related to how well the tree can thrive in its environment.

Tree diagnoses deal with specific problems that may occur through either biotic or abiotic factors. You will look for damage symptoms that have been caused by a variety of different agents. A symptom is something you notice which may tell you that the tree is being damaged. Tree symptoms can be used in the same way that a doctor uses an individual's coughing and nose blowing as symptoms to diagnose a cold. The cold (virus infection) is the agent for the symptoms of the coughing and runny nose! You will look for agents of damage and try to figure out if they are affecting the whole tree and/or other trees in the PSSP.



Examples of Stem Form

Discussion Questions

1. What is tree form?
2. How can tree diagnosis observationshelp us determine the general conditions of the forest?

TREE FORM AND DIAGNOSIS

PROTOCOL

How can we assess each sample tree for its overall form and to diagnose its health?

Materials

- Tree Form and Diagnosis Data Sheet

In this protocol, you will assess the form and make a health diagnosis of each of the permanently marked trees in the PSSP.

Procedure

1. Carefully fill out your tree form and diagnosis data sheet as you observe your five white pines. Be sure to look at all aspects of the tree: roots, bark, branches, crown, leaves, etc.
2. Compare your observations for your five white pines to other trees in your PSSP. Note how similar or different they are in terms of form and apparent health.

TREE FORM AND DIAGNOSIS DATA SHEET

Name:_____ Date:_____

School:_____ Teacher:_____

1. In the spaces below, enter the tree # and use the key provided to best describe tree form:

Be sure to note if the tree is no longer standing, dead or dying.

Tree #: _____ Tree Form: _____

Sketch & Notes:

Tree #: _____ Tree Form: _____

Sketch & Notes:

Tree #: _____ Tree Form: _____

Sketch & Notes:

Tree #: _____ Tree Form: _____

Sketch & Notes:

Tree #: _____ Tree Form: _____

Sketch & Notes:

2. Describe the location of your PSSP.

3. Observe each tree and check the appropriate spaces in the table below:

	Tree#	Tree#	Tree#	Tree#	Tree#
Needles					
missing:					
yellowing:					
wilted:					
spotted/mottled:					
unusual characteristics:					
damage(describe):					
	Tree#	Tree#	Tree#	Tree#	Tree#
Main Stem					
oozing sap:					
insect hole/damage:					
woodpecker holes:					
other animal damage:					
(describe)					
loose bark:					
fungi,galls,cankers:					
visible rot:					
lightning damage:					
other damage: (describe)					
	Tree#	Tree#	Tree#	Tree#	Tree#
Main Stem					
broken:					
missing foliage:					
many dead:					
	Tree#	Tree#	Tree#	Tree#	Tree#
Roots					
exposed:					

4. Is there any evidence of lichens, moss or fungi on the trees? Which trees are affected?

5. How does the tree form and diagnosis of the tagged trees compare to the other trees in the PSSP?

6. Have trees in your forest stand been affected by a recent storm such as an ice storm or high winds? Explain.

7. Note any other observations on the trees.

TREE SELECTION AND TAGGING

PROTOCOL

How will you choose and tag sample trees for the Forest Watch study?

Once you have established and marked the corners of your pixel-sized sampling plot (PSSP), you are ready to choose the five white pine trees that you will use for the Forest Watch study. You, and many students in the years to follow, will conduct several measurements using these trees and their needles. These trees will be permanently tagged with a numbered aluminum tag and nail. No other trees will have the same identification numbers and they will become a permanent part of the Forest Watch database. Using the same trees year after year will enable researchers to conduct change-over-time analyses and will help with the understanding of how your forest may be changing.

Materials

- numbered aluminum tags
- hammer
- metric measuring tape or stick

Procedure

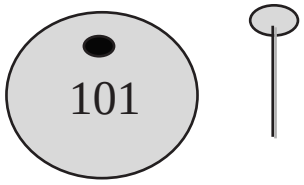
Selecting your trees

You need to select five trees that are in the main region of foliage; this is called the dominant canopy. You can think of the dominant canopy as those trees that receive sunlight first. Dominant trees are trees that should be the most functional under normal conditions of sufficient light, nutrients and water. They should lack any visible, large-scale affects from damaging agents.

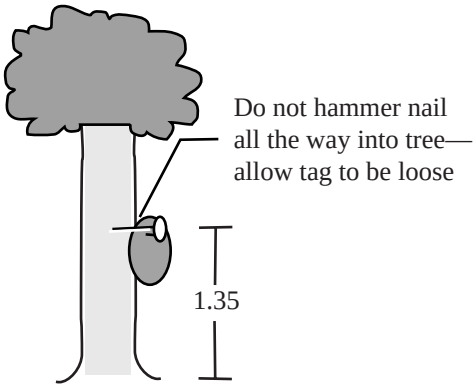
1. Identify the white pine trees available for study in your PSSP.
2. Use the following criteria to help you select the five trees best suited for your study:
   a. Select a range of tree sizes, for example, two shorter, one medium and two taller trees.
   b. Make sure you will be able to collect foliage samples from the trees you choose. You will be using a pole pruner that will reach about 10 meters or so up into the actively growing canopy.
   c. You should choose trees that have good form and look relatively healthy.
   d. You should choose trees which are representative of your stand.

Permanently tagging the trees

1. Use the metric tape or ruler to measure a height up the tree 1.35 m (4.5 ft) from ground level. This is the height at which you will take DBH (diameter breast height) measurements in the future.
2. Hammer the nail on one side of the tree: north, south, east or west. Make sure that you leave the nail head at least 2-3 cm out of the tree. This will allow room for the tree to grow. Every year or so your teacher will pull the nail out a little to keep ahead of the tree's growth.
3. Repeat this procedure for the remaining four trees, always placing the tag at a height of 1.35 m from the tree base and facing the same cardinal direction.



Tree Identification Tag & Nail



Location of Tag on Tree

TREE SELECTION AND TAGGING

TEACHER RESOURCE

Introduction

Once you have established and marked the corners of your pixel-sized sampling plot (PSSP), you are ready to choose the five white pine trees that you will use for the Forest Watch study. You, and many students in the years to follow, will conduct several measurements using these trees and their needles. These trees will be permanently tagged with a numbered aluminum tag and nail. No other trees will have the same identification numbers and they will become a permanent part of the Forest Watch database. Using the same trees year after year will enable researchers to conduct change-over-time analyses and will help with the understanding of how your forest may be changing.

Materials

- numbered aluminum tags
- hammer
- metric measuring tape or stick

Management Suggestions

This protocol is a **one-time only protocol** completed during the first season of your study or, if for some reason, you need to select a new tree or trees.

Make sure you have permission from the landowner to permanently tag the trees and he/she does not plan to cut any trees on the PSSP.

The tags should be hammered into the tree at the position that your DBH measurements will be taken: at 1.35 m (4.5 ft). It is best to place the tags all facing the same cardinal direction (e.g., north) to assist you in relocating these trees on successive field visits. An aluminum tree nail should be used (provided with the tag) so in the event that the tree is ever milled, the nail will be soft enough so that it doesn't ruin a saw blade or bind-up a chain saw (which could cause serious bodily injury to the operator). Additionally, the nail should not be hammered all the way in so that it is flush against the tag—*leave the nail head 2-3 cm out of the tree!* This will allow room for the tree to grow. If the nail were all the way into the tree the tree would grow around the tag and nail, and within a couple of years you would not be able to find the tag.

If for some reason you should need to change trees (for example, if one dies or you can no longer reach the foliage for samples), please be sure to contact us at UNH to let us know. We will issue you a new tree number. **Do not** reuse the same tree number from one of your older trees! If you need to add a new tree because you can't reach the needle samples on an old tree, please continue to collect information from the old tree regarding diameter and height.

Another helpful piece of information to have about each of your trees is tree morphology. We highly suggest that you conduct the Tree Form and Diagnosis Protocol for each of your trees and send that information to us at UNH. Additionally, take photos of each of your trees as well as photos of your plot. To take photos of your plot, stand at each corner and face the center of the PSSP. Be sure to label the location, your school name, the teacher name and the date the photo was taken; send copies of these to UNH. If you have any questions regarding the selection of trees for your study plot please contact us at UNH.

COLLECTING WHITE PINE NEEDLE SAMPLES AND SENDING THEM TO UNH

TEACHER RESOURCE

How do we collect needle samples from white pine trees?

How should these samples be sent to UNH?

Introduction

Needle samples will be collected, each year, from the north and south quadrants of your five white pines. Some of the needles will be kept for your study at school and some of the needles will be sent to UNH for spectral analysis. It is important to make sure that your needles are stored in closed, zip-loc bags containing moist, paper towels. Keep the bags in a refrigerator between classes. During class time, it is helpful to have a large cooler placed in a central location where your students can retrieve needles as necessary, yet keeping the bags in a cool place.

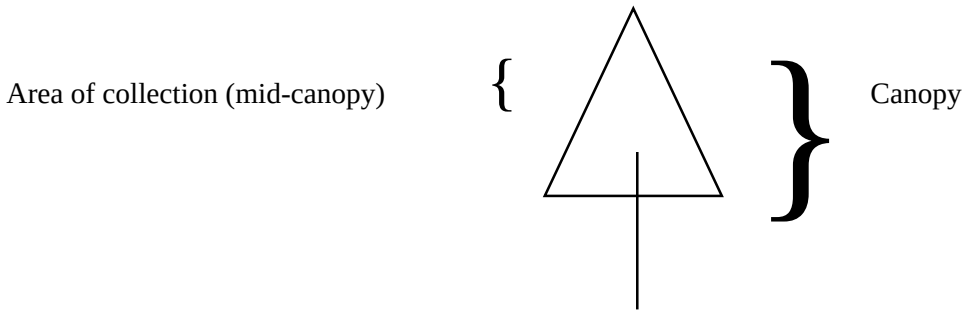
Materials

- pole pruners
- compasses
- large cooler for the field
- frozen ice packs
- 20 large zip-loc bags
- permanent markers
- small mailing cooler from UNH

Procedure

Obtaining needle samples

Use pole pruners to cut branch samples from the outside edges of the mid-canopy of the north and south quadrants of each tree. The pole pruners can be fairly tricky to use, so be sure to have several people helping. Ask students to stand away from where the pole pruners might fall if you should lose your strength or stamina while holding the pole pruners up! When extensions are added the pole can become heavy and quite tricky to maneuver. It may be a good idea for you and your students, if they will be cutting, to practice using the pruners on other trees first. Be careful not to cut too much though!



As the branches are being cut, make sure there is a student responsible for determining and immediately recording the height from which the samples have been taken. This can be done easily if you know the length of the pole and the height it is being lifted off the ground at the time of cut. Record this data now on the Tree Height Data sheet found in the Tree Height Protocol.

When the branches fall to the ground, have a couple of students pick them up and prepare them for bagging. Some of the needles will be kept in bags for your use and some of them will be sent to UNH. Quickly divide the needles and place samples in prelabeled zip-loc bags that also contain a moist paper towel. The bags should be labeled as follows:

- school name
- tree #
- quadrant (N or S)
- date of collection

You will need to send at least five bunches of samples to UNH so a complete scan can be made. Bunches include a group of first-year needles that are still attached to the stem by fascicles. **Make sure that you send plenty of first-year needles that are still attached to the branch.**

Place both bags into the large cooler that you have brought to the field with frozen ice packs and move on to the next quadrant. Repeat this procedure for each of your trees. You will end up with two duplicate sets of bags. Do not let the samples sit around unbagged and do not let the bags sit around outside the cooler. It is very easy to confuse samples after they have been cut if they are not immediately placed in labeled bags. And they will begin to degrade and dry out if they are not quickly placed in the cooler.

Sending Samples to UNH

In the lab, prepare your bags for long-term storage by checking to make sure there are no punctures in your bags and that the paper towels are still moist. Close the bags while squeezing out as much air as possible.

Place the ten bags for UNH into the small cooler, provided by UNH, with a small ice pack. Use a pencil to carefully fill out a Forest Watch Sample Submission Sheet and include it in the top of the cooler. The submission data sheet is critical to have because it speeds up the scanning process at UNH and increases accuracy and efficiency.

Store the cooler in a refrigerator until you are ready to send it to UNH. You may send the cooler by First Class mail. You may also send it by UPS or FedEx, if your school can afford to do so. Samples should be sent out on a Monday, Tuesday or early Wednesday morning. If you collect on a Wednesday afternoon or on a Thursday or Friday, keep the coolers in a refrigerator over the weekend and send out the following Monday morning. Samples are not safe sitting in a warm truck or post office over the weekend.

FOREST WATCH
FRESH SAMPLE SUBMISSION SHEET

Please Fill Out this Form and Submit it with your Cooler

School Name _____

Tree #'s _____ Address _____

Teacher_____ _____

Date Samples Collected _____ _____

Checklist:

Phone #: _____

Wet paper towel in each bag? _____ Email: _____

School, Tree #, and location (N vs. S) on each bag?_____

Blue Ice? _____

Please note any special circumstances:

Note: First Class/Priority Mail is generally sufficient for shipping, if mailed on Monday or Tuesday. Try to avoid sending us needles on Thursday or Friday; instead keep them refrigerated until the following week. As long as needles are connected to the branch sample and are kept cool and moist, sealed in a zip-loc bag, they will be ok for up to 1-2 weeks. But, the sooner we receive them the better.

TREE MEASUREMENTS

C

CONTENTS

TREE HEIGHT, LIVE CROWN HEIGHT AND SAMPLE HEIGHT

BACKGROUND

Guiding Questions

How can you measure the height of a tree?

Why are tree height measurements useful for this study?

One of the measurements you will make for the Forest Watch study concerns the height of your sample trees, the height of the actively growing portion of the tree called the live crown and the height at which you take your needle samples. Each year you may notice a change in the tree and live crown height. Changes in height may help you assess the way your tree is growing and can be used to estimate stand biomass and interpret satellite data.

Sample collection height helps you monitor whether you have sampled in the live crown or perhaps below it in the less vigorous portion of the tree. Knowing the sample height can help you interpret the results from other portions of your study. For example, if you sampled needles from the shaded lower portions of the canopy, your results from needle assessment measures might indicate needles of poor health. These results would not be a true measure of the active portion of the canopy.

Tree height is a measure of how tall a tree is from its base to the very top. In a uniform, even-age stand of white pine trees, the relative heights of the trees in the stand may be an indicator of tree health. Placement within a stand will also affect how a tree grows as the vegetation competes for nutrients, water, space and sunlight. Most of the white pines in the Forest Watch study are probably not found in uniform, even-age stands but instead in mixed stands or as individual trees growing near your school. You will use measures of tree height, and how it changes over time, to help characterize a general pattern of growth. Over time, stresses on the trees such as drought, insect infestation or poor air quality could result in a slowing of tree growth. Knowing the height of the trees in relation to others around them will give you a visual image and insight into the environment in which the tree is growing.

Live crown height is a measure of the actively growing branch and needle section of the tree. The live crown extends from the top of the tree to the base of the living portion of the canopy. The branches at the base of the live crown of a mature white pine will generally be dead or nearly dead. Needle growth is sparse or totally absent on these lower branches as the sunlight is shaded from above. Trees in which a large proportion of the crown has healthy green needles have a high photosynthetic potential and may therefore show more growth from year to year than adjacent trees with less needle mass and/or chlorotic (yellow) needles.

Sample height is the height of the point on the tree from which your samples were collected. Try to collect your samples from the middle of the live crown. It is important to sample from both the north and south quadrants (sides).

Discussion Questions

1. List the three measurements (tree, live crown and sample) in order of increasing height from the ground.

2. How might these measurements change over time?

3. What factors might cause the changes discussed in question.

MEASURING TREE HEIGHT, LIVE CROWN HEIGHT AND SAMPLE HEIGHT

C

PROTOCOL

How can you measure tree height, live crown height, and sample height?

Materials

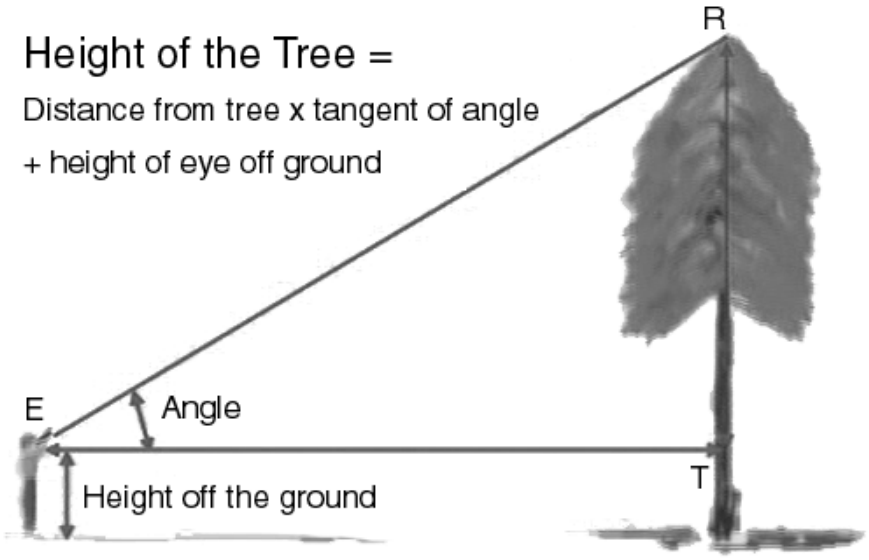
- clinometer
- pole pruners or some other similar sampling device
- field tape measure (metric)
- data sheets

Procedure

You will work with a group of students on this activity. Each group should repeat the measurements for a total of three trials. Record the data on the data sheet provided.

A. Measuring Tree Height Refer to the illustration below.

1. Select a location that is far enough away from your tree so that you can see the treetop. The further away from the tree you are, the more accurate your measurement of its height. This distance represents the baseline distance equal to line ET on the diagram below and can be measured with a tape or by pacing.
2. Mark your location (E) with a flag or stake so that additional measurements by your partner(s) can be made from this point.



3. Use the clinometer to obtain the angle measurement (∠RET) created by the baseline and your sight line to the top of the tree. Stand at the marked location (E) and hold the clinometer up to your eye so that you can look through the end of the straw opposite the string. While looking through the straw, sight to the top of the tree (R). Allow the string and weight to hang freely. Once you have the top of the tree in view through the straw, have your partner hold the string steady against the clinometer. Your partner should read the angle on the clinometer where the string crosses the scale. You can now use this angle to find the height of your tree.
4. Use the tangent table to look up the tangent of ∠RET.
5. Measure the observer's height from the ground to his or her eye.
6. Record all your information on the data table and use it to determine tree height.
7. Repeat procedures 3 three times with other people in your group. Average your results.

B. Measuring Height to the Base of Live Crown to determine the Average Live Crown Height

The base of the live crown is the bottom point of a tree's crown where the first dominant live branch(es) and its needles occur. Below the live crown, a white pine may have dead branches or nearly dead branches with some needles, but no vigorous growth. Do not include these dead branches as part of the live crown.

You can also determine the height at the base of the live crown by using the same method that you used to find tree height. Remember, however, that you should sight to the base of the crown not to the top of the tree.

Now use the data for tree height and height to the bottom of the live crown to determine the height of the live crown portion of the tree. Record your data on the data sheet.

In some cases, you can verify your clinometer measurement to the bottom of the live crown by using the known length of the pole pruner sections. Add pole sections until the length of the pruner extends from the ground to the base of the live crown. Calculate the height to the base of the live crown and record it in the data table.

C. Measuring Sample Height

To measure sample height, use the length of the pole pruners to find the height at which you have collected your samples. Find this height at the same time as you are collecting your sample from both the north and south quadrants.

TREE HEIGHT, LIVE CROWN HEIGHT AND SAMPLE HEIGHT DATA SHEET

Students/Class:_____ Date:_____

School:_____Teacher_____ Town: _____

Tree #: _____ Site location:_____

A. Measuring Tree Height (record to the nearest 1.0 m)

	Trial 1	Trial 2	Trial 3
Baseline distance (m)	_____	_____	_____
Clinometer Angle	_____	_____	_____
Tangent of Angle	_____	_____	_____
Height of Eye to Ground (m)	_____	_____	_____
Height of Tree (m) (Tangent x Baseline) + Eye Height	_____	_____	_____

Average Tree Height (m): _____

B. Measuring Height to Base of Live Crown (record to the nearest 1.0 m)

	Trial 1		Trial 2		Trial 3	
	North	South	North	South	North	South
Baseline distance (m)	____	____	____	____	____	____
Clinometer Angle	____	____	____	____	____	____
Tangent of Angle	____	____	____	____	____	____
Height of Eye to Ground (m)	____	____	____	____	____	____
Height to Base of Live Crown (m) (Tangent x Baseline) + Eye Height	____	____	____	____	____	____

Average Height to Base of Live Crown (m): _____

Average Live Crown Height (m): _____

(Average Tree Height - Average Height to Base of Live Crown)

C. Measuring Sample Height (record to nearest 1.0 m)

North Quadrant	Trial 1	Trial 2	Trial 3
Baseline distance (m)	_____	_____	_____
Clinometer Angle	_____	_____	_____
Tangent of Angle	_____	_____	_____
Height of Eye to Ground (m)	_____	_____	_____
Height of Sample (m) (Tangent x Baseline) + Eye Height	_____	_____	_____

(Average) North Sample Height (m): _____

South Quadrant	Trial 1	Trial 2	Trial 3
Baseline distance (m)	_____	_____	_____
Clinometer Angle	_____	_____	_____
Tangent of Angle	_____	_____	_____
Height of Eye to Ground (m)	_____	_____	_____
Height of Sample (m) (Tangent x Baseline) + Eye Height	_____	_____	_____

(Average) South Sample Height (m): _____

Analysis and Interpretation

1. How do the heights compare for each of the 3 trials your group made of your tree?

2. In some cases, the heights that are measured in each of the three trials may not all be the same. Speculate as to what factors might cause differences in the measures that students make for the same tree.

3. If you have observed a lot of variation in your measurements between the trials, what suggestions can you make for improving your technique?

4. What was the height of your tree last year? How does this compare to your current measure?

5. Is the tree losing its live crown as compared to last year's measurement?

6. Brainstorm some ideas as to why tree height measurements might decrease from one year to the next?

CLINOMETER CONSTRUCTION

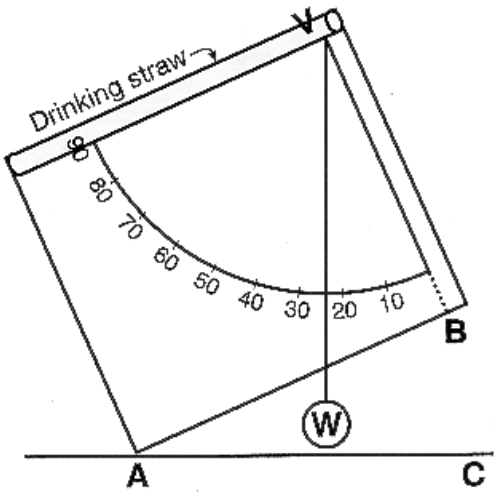
C

Materials

- Piece of string approximately 20 cm long
- Small washer (or some kind of weight for the string)
- Large plastic straw
- Piece of cardboard
- Clinometer template
- Tape and/or glue
- Scissors
- Laminating film
- Tangent table

Procedure

1. Study the diagram and follow the directions to construct a clinometer.
2. Carefully cut out both the clinometer pattern and the tangent table box from the handout you've been given by your teacher. Tape or glue the pieces to either side of a piece of cardboard that measures just slightly larger than the clinometer/tangent table pattern.
3. If possible, laminate the cardboard clinometer for extra protection.
4. Make a <u>small</u> hole at the mark labeled **V** in the corner of the cardboard clinometer. Use a pencil, pen point or tip of scissors.
5. Lay the string along the right margin of the clinometer below the hole you've just made. Pull a short length of string through the hole and make a large knot in the string on the back side of the clinometer. Make sure that the string is still longer than the cardboard.



6. Tie the washer **(W)** to the free end of the string. Make sure that the string still swings freely and the washer is below the clinometer scale at all points.
7. Attach the drinking straw with clear tape and/or glue along the upper margin of the cardboard clinometer. <u>Make sure that you attach the straw exactly on the indicated line or your angle measures will not be correct</u>.

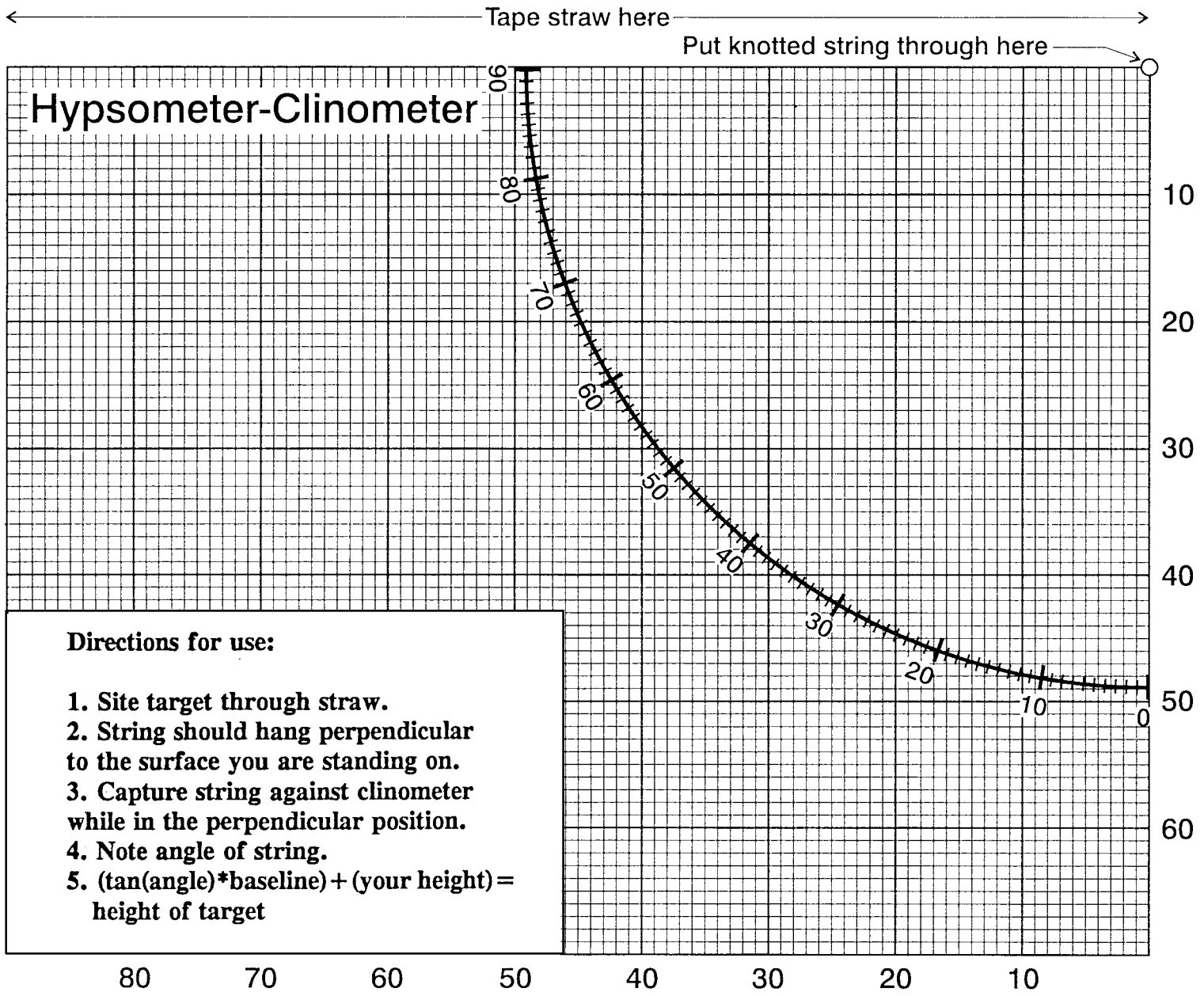
We also need a clinometer template placed after GS 4.

Table of Tangents

Angle	Tan.	Angle	Tan.	Angle	Tan.	Angle	Tan.	Angle	Tan.
1°	.02	17	.31	33	.65	49	1.15	65	2.14
2	.03	18	.32	34	.67	50	1.19	66	2.25
3	.05	19	.34	35	.70	51	1.23	67	2.36
4	.07	20	.36	36	.73	52	1.28	68	2.48
5	.09	21	.38	37	.75	53	1.33	69	2.61
6	.11	22	.40	38	.78	54	1.38	70	2.75
7	.12	23	.42	39	.81	55	1.43	71	2.90
8	.14	24	.45	40	.84	56	1.48	72	3.08
9	.16	25	.47	41	.87	57	1.54	73	3.27
10	.18	26	.49	42	.90	58	1.60	74	3.49
11	.19	27	.51	43	.93	59	1.66	75	3.73
12	.21	28	.53	44	.97	60	1.73	76	4.01
13	.23	29	.55	45	1.00	61	1.80	77	4.33
14	.25	30	.58	46	1.04	62	1.88	78	4.70
15	.27	31	.60	47	1.07	63	1.96	79	5.14
16	.29	32	.62	48	1.11	64	2.05	80	5.67

Example: Assume you have established a baseline distance of 60 meters. Assume that you have measured the tree top to an angle of 24°. From the table, you will see that the tangent of 24° is 0.45. Therefore, the tree height is 60m x 0.45 = 27 meters. By adding the height of the eyes of the observer (1.5m), the total tree height is 28.5 meters.

Example: Working from a sample baseline distance of 60 meters and a treetop angle measurement of 240, find the tree height as follows: from the table, you will see that the tangent of 240 is 0.45. Therefore, the tree height from the eye of the observer is 60m x 0.45 = 27 meters. By adding the height from the observer's eyes to the ground (e.g. 1.5 m), the total height of the tree is 28.5 meters.


Tape straw here
Put knotted string through here
Hypsometer-Clinometer
90
80
70
60
50
40
30
20
10
0
10
20
30
40
50
60
80
70
60
50
40
30
20
10
Directions for use:
1. Site target through straw.
2. String should hang perpendicular to the surface you are standing on.
3. Capture string against clinometer while in the perpendicular position.
4. Note angle of string.
5. (tan(angle)*baseline)+(your height)= height of target

TREE HEIGHT, LIVE CROWN HEIGHT AND SAMPLE HEIGHT

TEACHER RESOURCE

Introduction

In this activity, students will use a hand-constructed or traditional clinometer (obtained from a forestry supply catalog) to determine three height measurements on their sample trees. They will measure total tree height, height of the live crown and height where the needle sample was collected. In addition to the clinometer, a table of tangents and simple math skills will allow the determination of the heights and will provide an excellent example of how math is used in science. No detailed knowledge of trigonometry is necessary for either the instructor or the student, however this could be a great opportunity to show your students this math tool.

Guiding Questions

How can you measure the height of a tree?

Why are tree height measurements useful for this study?

Materials

- clinometer
- sampling device such as a pole pruners
- field tape measure (metric)
- data sheets

Background

Tree height is useful for an overall characterization and mental image of your study site. Tracking these measurements year after year should allow you to observe tree growth over time.

Live crown height is important because it indicates the area of the most actively growing portion of the tree relative to the tree's total height. The live crown extends from the top of the tree to the base of the living portion of the canopy. The branches at the base of the live crown of a mature white pine will generally be dead or nearly dead branches with a few green needles, but no vigorous growth. The larger the live crown relative to tree height, the greater the photosynthetic capability of the tree. The ability to photosynthesize over a large portion of the tree may ultimately affect tree health.

Sample height is the location in the live crown where the Forest Watch samples are collected. It is important to collect your samples from the actively growing live crown. Samples are collected from both the north and south sides (quadrants) of each tree.

Management Suggestions

Practice making and using the clinometer to measure tree height before doing this activity with your students. Small student groups work well both in making and using the clinometer to determine the various height measurements.

If time is a constraint, you might consider locating and marking known baseline distances from your sample trees before the class goes out to the field. This could improve accuracy in your measurements and speed up the process by allowing for more trials to be conducted in the time available to you.

Student groups should complete at least 3 trials for each angle measurement on their tree and compare the height values they have calculated. If student measurements vary significantly, they should repeat their measurement until a consensus is reached. If you have measurements from previous years' classes, have your students compare their results with past heights to further troubleshoot for measurement errors.

Clinometer Construction

Each group of students should have at least one clinometer available to them to efficiently complete the height measurements. Use the templates for the clinometer and tangent table provided with this protocol to make your own instruments or purchase professional clinometers from a forestry supply catalog. Clinometer prices may vary from relatively inexpensive ($15) to very expensive models. If constructed and used carefully, however, you should feel confident in the accuracy of your homemade instrument.

Additional Suggestions

Class discussion may be used to help students make the connections between height measurements and tree/forest health. You will need previous years' data to discuss some of the following questions.

1. How do live crown sizes of the 5 trees compare?
2. How has crown size changed over time?
3. How high were your samples collected within the live crown and why is it important to collect samples in the middle of the live crown?
4. What percentage of the tree height is live crown?
5. What is the average tree height for all 5 trees?
6. What is the average crown height and why would we want to know this information?
7. Do your trees represent the height of most trees in your study site?
8. What conclusions might you now draw about your forest stand?

Using data from the Class Data sheet, ask your students to prepare a histogram of tree height, sample height, and height to the base of the live crown. Include a key and label the sample height for the north and south quadrants. Students may wish to prepare and compare graphs of previous years' data as well. See the Math Activities section for details and examples.

Have a student group take measurements of other tree species in your forest stand and prepare histograms like those used for your white pine trees. Compare white pine trees to other tree species within the stand. Perhaps you could speculate about the stage of succession that the forest plot is now in. See the introductory activity on succession for further details.

Answer Key

Discussion Questions

1. List the three measurements (tree, live crown and sample) in order of increasing height from the ground.

   From the ground, first is the base of the live crown, then sample height, followed by the top of the tree.

2. How might these measurements change over time?

   The base of the crown should rise in height as the lower, older branches die off, and the tree itself grows taller. One would expect that a healthy tree, in its actively growing years, will increase in height each year. Presumably, there must be some maximum height that a typical mature white pine tree will reach as it ages. If environmental conditions are causing stress on the forest or to the tree itself, tree growth might be affected.

3. What factors might cause the changes discussed in question #2?

   Growth may be affected by natural or human events. Positive factors include loss of competing flora, favorable climatic conditions, etc. Negative factors may include competition for light, moisture, nutrients and space from neighboring plants and/or other damage caused by natural (e.g. insect) or human (e.g. pollution) factors.

Analysis and Interpretation Questions

1. How do the heights compare for each of the 3 trials your group made of your tree?

   The intent is to have students look critically at the variation in their measurements. Ideally, they should all be identical.

2. In some cases, the heights found in each of the three trials may not all be the same. Speculate as to what factors might cause differences in the measures that students make for the same tree.

   Some of the reasons for differences might be terrain interference in measurement, human error in the use of the measuring equipment, and errors in mathematical calculations.

3. If you have observed a lot of variation in your measurements between the trials, what suggestions can you make for improving your technique?

   Find a more suitable position in the surrounding terrain from which to take the measurement, more practice and care in the use of equipment and the taking of measurements and more precision in making the mathematical calculations. Encourage students to look at data from other groups who measured the same tree. Looking at the variation in other data sets may help them to make a judgment about their own measurements.

4. Brainstorm some ideas as to why tree height measurements might decrease from one year to the next?

   Reasons could include: student error in measurement or calculations, insect damage, weather damage (lightning strike, wind, etc.) and disease.

DBH (TREE DIAMETER) MEASUREMENTS

BACKGROUND

Guiding Questions

How can we accurately measure the growth rate of a tree?

Why are tree diameter measurements useful for this study?

Foresters use a measurement of a tree's diameter called DBH or diameter at breast height. Tree diameter is an important forestry measure and is used to indicate how well a tree is growing over time. It is also one of the standard measures of timber volume used to estimate the commercial value of a forest stand. By convention, the diameter is measured at a height on the trunk that is 1.35 m (4.5 ft) above ground level. This height above the ground is used because uneven swelling and irregular growth at the base of the tree and upper roots could mask the true growth of the trunk.

For the Forest Watch study, DBH will serve as a yearly measure to help characterize the growth of your white pine trees. Measurements must be taken at exactly the same height on the trunk each year. Otherwise, teachers and students will find that their tree diameters may shrink or make inaccurate jumps in width from year to year!

Discussion Questions

1. There are cases where a stand of white pine trees is all the same age, but the diameters of these trees seem to vary in size. What might be some of the reasons why these trees have different diameters?

2. How might tree diameter, when viewed over several years, be related to tree health?

DBH (TREE DIAMETER) MEASUREMENTS

PROTOCOL

How do you measure the DBH of a tree?

Materials

- metric or forestry DBH tape measure
- DBH data sheet
- calculator

Procedure

You will work with at least one partner for this protocol, taking and recording 3 measurements of tree circumference.

1. Locate the point on the tree trunk that measures exactly 1.35 m from ground level. Carefully and evenly, wrap the tape measure around the tree trunk at the 1.35 m mark. Your partner should stand in the back of the tree to make sure that the tape is horizontal all the way around.
2. Read the circumference at the point at which the tape crosses over its starting point. Make sure you are reading the tape correctly.
3. Record the measurement to nearest centimeter on your DBH Data Sheet.
4. Measure and record the tree circumference two more times.
5. Using the formula: $D = C/\pi$, calculate and record the diameter for the tree on the DBH Data Sheet.

If you are using a forestry DBH tape you will not need to calculate diameter by using the formula in step 5. The tape is already calibrated to read diameter. Simply record the value at the point at which the tape crosses over its starting point as the diameter in centimeters. Be careful to read the correct side of the tape. One side has normal metric measures and the other measures diameters. They are clearly marked at the end of the tape. Measure by matching the vertical line at the end of the tape with the diameter measurement. Watch for the sharp hook!

DBH DATA SHEET

Students/Class:_____ Date:_____

School:_____ Teacher_____ Town:_____

Tree #:_____ Site Location:_____

CIRCUMFERENCE READINGS: or DIRECT DIAMETER READINGS

Trial 1 circumference = _____cm

Trail 2 circumference = _____cm

Trial 3 circumference = _____cm

Average circumference =_____cm

Trial 1 DBH = _____cm

Trail 2 DBH = _____cm

Trial 3 DBH = _____cm

Average DBH =_____ cm
(record to the nearest 0.1 cm)

Average DBH = _____ cm ÷ 3.14
(Use formula: DBH = C/π and record to the nearest 0.1 cm)

CLASS DATA FROM ALL TREES

Collect each group's DBH data and record in the space below:

Tree #	This year's DBH (cm)	Last year's DBH (cm)	Amount of growth (cm)
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

Analysis and Interpretation

1. Why is it always important to follow protocol instructions exactly as stated?

2. What relationship might exist between tree DBH and tree height for the same tree?

3. Construct a bar or line graph of the DBH data for three trees in a sample study plot gathered over a five year period. Use the sample data below.

DBH Measurements (cm) for three trees from 1993-1997

	Year				
	1993	1994	1995	1996	1997
Tree #					
1	29	34	36	39	41
2	36	39	41	44	46
3	34	31	34	32	33

4. Describe the growth patterns you observe for each tree in terms of DBH. Provide an explanation for the patterns you have described.

DBH (TREE DIAMETER) MEASUREMENTS

TEACHER RESOURCE

Introduction

In this activity, we are measuring the diameter of the sample trees using the conventional forestry measurement called DBH (diameter at breast height). This annual measurement is taken at a height of 1.35 meters up the tree trunk from ground level.

Guiding Questions

How can we measure the diameter of a tree?

Why are tree diameter measurements useful for this study?

Materials

- metric or forestry DBH tape measure
- DBH data sheet
- calculator

Background

Foresters use a measurement of a tree's diameter called DBH or diameter at breast height. Tree diameter is an important forestry measure and is used to indicate how well a tree is growing over time. It is also one of the standard measures of timber volume used to estimate the commercial value of a forest stand. By convention, the diameter is measured at a height on the trunk that is 1.35 m (4.5 ft) above ground level. This height above the ground is used because uneven swelling and irregular growth at the base of the tree and upper roots could mask the true growth of the trunk.

For the Forest Watch study, DBH will serve as a yearly measure to help characterize the growth of your sample trees. Measurements must be taken at exactly the same height on the trunk each year. Otherwise, teachers and students will find that their trees' diameters may shrink or make fantastic jumps in width from year to year!

Management Suggestions

Placing your students into groups or teams works well when doing this activity. Generally, each team will study one of your school's white pine samples and may continue working in the same groups throughout the entire study. A teacher may select other arrangements to suit his/her needs.

If available, use a metric tape measure for this activity as all data are to be recorded using the metric system. Otherwise, students will have to convert English standard measures to metric using conversions such as 1" = 2.54 cm or 12" = 30.48 cm. This exercise could provide valuable review and practice in conversion.

Mark your tree at exactly 1.35 m from ground level for future measurements

A. Try placing the numbered tree identification tags at exactly 1.35 m above ground level when you first tag your trees. Be sure to check the bark for projecting branches or old knots. Hammer the tag into the tree leaving about a 2-3 cm space behind the tag to allow for annual trunk growth. Each year the tag should be checked to see that there is enough growth space behind the tag. Use a hammer to pull the nail out a little bit if necessary.

or

B. Make a mark on one of your pruning poles 1.35 m from an end, stand it beside your tree and mark the breast height as noted above.

Additional Suggestions

The following activity may be useful for students who have not had much work with the formula: $D = C/\pi$. Try it before going out into the field.

Bring to class various types of round fruit such as oranges, grapefruits, or peaches. Place several potential measuring tools at stations around the classroom such as a metric tape, metric ruler, barbecue skewers, pencils, and string. Then present the following scenario. "Our class has been asked to pack the fruit you've been given into the smallest possible size box for shipment. What information about this fruit would help us accomplish that goal?" Students might suggest massing or weighing the fruit, what kind of packing material will be used, how fresh is the fruit, what is the size of the fruit, etc. Point out that size of the fruit is one of the most important things to know. "What are some ways that we could determine the size of the fruit?" Students might suggest set it down on top of a ruler and measure side to side, poke a hole in it and measure how far through a skewer might go, etc.

Allow students to discuss the possibilities but present the rule that the fruit must remain in tact. After students have determined an answer, introduce the mathematical formula that can be used to determine the diameter of an object: $D = C/\pi$. Work students through several examples of objects in the room.

If a cross section through the trunk of a tree is available this would be an ideal time to use it in the explanation. Explain that using the formula is important when we do not want to damage or poke holes in an object such as a living tree or fruit to be crated and sent to a grocery store. Have students try an example on their own.

Guide students through a discussion on the accuracy of their measurements. Is the circumference reading exact? Was it measured exactly in the middle of the fruit? Did the string or tape slip while you were trying to hold it? Should you take more than one measurement? Why?

For Further Study

1. Once students have done other Forest Watch measurements, they can compare them to DBH to see if a change in one measure signals a change in another: either larger or smaller. Is there a relationship between DBH and tree height, or between DBH and needle length for example? Remember, these changes are not necessarily cause and effect.
2. As your data base grows year after year, you can begin to look back at previous measurements for comparison. Students can look at change over time and check for accuracy in their measurements.

Answer Key

Background Discussion Questions

1. What might be some of the reasons why these trees have different diameters?

   Trees can be the same age and different sizes if they are of different species, if they are growing in different environments (more or less water, light, or soil nutrients), or if they are being negatively affected by insects, disease, or human factors etc.

2. Is tree diameter (DBH), when viewed over several years, related to tree health?

   If a steady increase in tree DBH is observed, the tree is generally considered healthy. Slower growth patterns may indicate environmental stress, while faster growth may result from less competition from surrounding vegetation.

Analysis and Interpretation Questions

1. Why is it always important to follow protocol instructions exactly as stated?

   If the protocol instructions are not followed errors in data collection may occur. In the case of DBH, these errors may result in a tree appearing to lose or gain excessive size from one year to the next. This erroneous information may then lead you to reach the wrong conclusion about the overall health of the tree.

2. What relationship might exist between tree DBH and tree height for the same tree?

   There should be a positive correlation in growth between the two variables. As tree height increases, one would expect to see an increase in DBH as well. Remember, this relationship is not one of cause and effect between the two variables.

3. Construct a bar or line graph of the DBH data for three trees in a sample study plot gathered over a five year period.

   The type of graph drawn can be either a line graph or a bar graph.

4. Describe the growth patterns you observe for each tree in terms of their DBH. Provide an explanation for the patterns you have described.

   Tree 1...Generally steady growth with the spike in 1993 possibly being due to unusually favorable growth factors.

   Tree 2...Steady growth pattern with little or no change in growth rate from year to year.

   Tree 3...Apparently erratic growth patterns, including 'negative' growth, possibly due to inaccurate measurements, possibly the result of failure to follow protocol instructions.

TREE MEASUREMENT DATA SHEET

Students/Class:_____ Date:_____

School:_____Teacher_____ Town: _____

Tree #: _____ Site location:_____

CLASS DATA: DBH (to 0.1 cm) and Tree, Live Crown, and N/S Quadrant Sample Heights (to nearest m)

Tree #	DBH (cm)	Average Tree Height (m)	Average Height to Base of Live Crown (m)	Average Live Crown Height (m)	Average Sample Height (m)
_____	_____	_____	_____	_____	_____N
					_____S
_____	_____	_____	_____	_____	_____N
					_____S
_____	_____	_____	_____	_____	_____N
					_____S
_____	_____	_____	_____	_____	_____N
					_____S
_____	_____	_____	_____	_____	_____N
					_____S

Transfer this data for the five white pine trees to the Master Data Sheet to be sent to UNH.

TREE MORPHOLOGY: COMPARING CONIFERS AND HARDWOODS

ACTIVITY

Can you relate tree morphology to tree and forest health?

Materials

- outdoor study site
- Tree Morphology Activity Sheet
- diagram(s) of tree morphology

Procedure

In this activity you and your partner will make a series of observations about the morphology (the physical characteristics) of two different types of trees. You will then try to relate the observed morphology to the overall tree and forest health.

1. Use the questions or statements in the left hand columns of the two sections that follow as a guide to filling in the Tree Morphology and the Tree Health tables for Tree #'s 1 and 2.
2. Answer the Analysis and Interpretation questions that follow.

TREE OBSERVATIONS: COMPARING CONIFERS AND HARDWOODS

Name(s)_____ Date _____

Site Location_____

Tree Morphology	Tree # 1	Tree # 2
Diagram and describe the shape of the crown (such as Christmas tree, round, oval, vase or "V" shaped)		
What is the color of the leaves in the crown relative to other trees?		
Select the word(s) that best describe the tree's trunk: Straight forked bent arched/curved		
Describe the texture (smooth, rough, furrowed, fissured) and color of the bark.		
Observe the tree's branching pattern. Sketch the tree trunk and 2 or 3 branches showing the angle at which the branches typically grow out of the tree.		
Describe the arrangement of the branches. Is the branching opposite, whorled, alternate?		
Observe how the needles or leaves are attached to the tree branch, draw or describe this arrangement.		

Tree Health	Tree # 1	Tree #2
Look for signs of damage. From the list below select the types of damage observed for each tree: Dead or dying trunk. Dead or dying branches or leaves. Yellowing leaves. Ice, wind or lightning damage. Broken or split trunk or branches. Bark damage by humans. Bark damage by animals (e.g. insects or beavers)		
From the list below select the types of organisms observed growing on each tree: Lichens Moss Bracket fungus None observed		
Does the area around the tree show signs of digging by animals or humans? Describe the extent of the digging.		

Analysis and Interpretation

1. What is your overall assessment of the health of the trees you have just studied? Justify your assessment using the observations you made.

   tree #1

   tree #2

2. Did you observe a relationship between tree form and tree health? Explain.

3. How might the health of an individual tree be ultimately related to the health of the forest in general?

Communicating your Analysis and Interpretation

After completing the Tree Observations worksheets, combine your assessment with those of several other classmates. Report your results to the class.

TREE MORPHOLOGY: COMPARING CONIFERS AND HARDWOODS

TEACHER RESOURCE

Introduction

In this activity, students will look at the general morphology or shape of a conifer and a hardwood tree in their PSSP. They will also make observations about tree health. By examining a number of trees, students can begin to make inferences about the overall health of the forest plot.

Guiding Question

Can you relate tree morphology to tree and forest health?

Materials

- outdoor study site
- diagram(s) of tree morphology
- Tree Morphology Activity Sheet

Background

Activities in this section will involve students in observing, writing, recording and drawing. They will use their observations to make inferences relating tree morphology to tree/forest health. Tree morphology includes all of the physical features associated with the tree. These include the general form and pattern of branching, the overall condition of its leaves and bark, etc.

Management Suggestions

Method and Procedures

Divide students into groups or teams to examine tree form. One of the trees the group observes should be the white pine tree that they will be sampling from later, although students do not necessarily need to know this at this time. This will serve as the initial, unbiased visual observation of the total tree. The tree form data can be an additional source as they collect more evidence about tree health from the protocols and other activities. In addition to assessing a white pine tree ask students to study a typical hardwood tree in your stand.

Posters and drawings can be created from the tree form data as part of a presentation at the end of the white study. Students may enjoy photographing their trees to get a visual record of change over time (See Site Assessment Protocols).

Time Frame

Students will need at least 25-30 minutes to complete the tree form activity sheet. Follow up discussion of the observations could be done in the field for the rest of the class period. This would present an opportunity for each group to be at the site when they report their findings. More time may be needed in the classroom the next day for discussion.

NEEDLE MEASUREMENT

C

CONTENTS

NEEDLE RETENTION

C

BACKGROUND

Guiding Question

How do you determine the number of years of needle retention on a white pine branch?

Each spring, white pines, and other trees in the forest, begin to take up water through their roots. This triggers a process in which the young buds at the tips of a pine branch begin to grow and a new set of needles develops. The buds, therefore, represent the future needle growth for the upcoming growing season. There are many, many small branches, all with maturing buds, found in the actively growing portion of the tree. Every year, the branches grow a little longer as a new set of needles matures.

All evergreen trees produce new needles every year. Some types, such as the tamarack, lose all of their needles every fall and grow new ones in the spring. Others keep several years worth of needles on their branches at one time. Hemlock, for example, may retain its needles for three to five years while spruces and firs could hold on to their needles for as many as fifteen years. A healthy Norway spruce may keep up to twenty years of needles.

Needles will remain on a branch as long as they can contribute to the overall growth of the tree. The needles are the photosynthetic organs on a conifer tree, and as long as a given age-class of needles remains photosynthetically active, they will be retained on the branch. Once the needles are no longer capable of photosynthetic activity, however, the needles will be cast (lost) from the branch. Air quality factors such as ground-level ozone will accelerate the aging process at the cellular level, resulting in loss of photosynthetic activity. Tree species that are sensitive to ozone will exhibit premature needle loss.

The term needle retention refers to the number of years worth of needle growth found on a pine tree branch. A white pine may have from one to four years worth of needles on its branches at the same time. Very healthy white pines may have needles from three or more growing seasons.

Needles are arranged in a variety of ways on coniferous trees. Some evergreens have needles that occur in clusters called fascicles while others, like the spruce, grow singly along the stem. White pine needles are grouped in a fascicle made up of five needles.

Discussion Questions

1. What are some factors, in addition to water, that probably cause a tree to begin to grow again in the spring in the temperate forest?
2. What do the buds represent at the end of a growing branch on a white pine tree?
3. Speculate as to why a tree might lose its needles at the end of a growing season even when it normally retains them for two or three years.
4. How do needles grow on the stem of a coniferous tree?

NEEDLE RETENTION AND FASCICLE LENGTH

PROTOCOL

How do you determine the number of years of needle retention on a white pine branch?

How do you measure fascicle length?

Materials

- pine branch morphology guide
- needle sample bag from the north or south quadrant
- Needle Retention and Fascicle Length Data Sheet
- millimeter ruler • calculator

Procedure

1. Select either the north or south sample bag from the cooler or refrigerator for your tree. Open the bag and remove one of the longer branches containing a length of healthy needles near its tip. Look along the length of the branch and make sure that the stem area near the bottom of the sample is bare. This portion of the stem has already lost needles from past years growth. Immediately close the bag and return it to the cooler or refrigerator.
2. Refer to the labeled diagram of a typical pine branch as you determine how many years growth can be found on your sample branch.
3. Find the terminal bud at the end of one of the growing tips on your branch. The bud at the end of the branch is the youngest growth on the branch. That bud contains all the new needles that will be emerging next June! You are concerned now, however, with the mature needles found below the bud along the length of the stem produced from last year's bud.
4. The needles found just below the bud are the needles that grew last summer. These are the needles that we will be sampling for our Forest Watch study. We will refer to them as current-year needles. They are now about one year old.

   One-year-old needles can be distinguished from two-year-old or older needles in several ways

   a. One-year-old needles should be just behind the terminal bud at the end of the branch.
   b. One-year-old needles may be lighter or brighter green in color.
   c. The bark of the stem that one-year-old needles are attached to may be a lighter brown color than the older stem segments found further down.
5. Successive-year needles are separated from one another by bud scale scars. Moving down the length of the stem and away from the bud, look for an area on the stem that resembles a set of fine rings running around the stem. This is the bud scale scar. The space between the scar is one year's worth of growth. There may be a correlation between the amount of stem growth (stem distance between buds and bud scale scars) and the growing conditions for that year.

6. Identify the number of years of needle retention on your sample branch and record on the needle data sheet.

   Now you are ready to randomly sample some needle fascicles and measure their length. Follow the directions below. Always select needle fascicles from the youngest mature needles on your branches, which grew and matured last summer.

7. Using the sample branch that you have been studying, locate several areas of growth where you find current-year needles. From one of the areas of current-year needle growth find a needle fascicle and gently remove it from the stem. Make sure that you leave the brown sheath which holds the bundle together intact.
8. Move from group to group of current-year needles and randomly select a total of ten needle fascicles varying where you take your samples from last summers growth. Place these fascicles on a piece of paper until you are ready to measure them.
9. Measure fascicle length in centimeters to the nearest 0.1 cm. You may notice that one or more of the five needles is shorter than some of the others. This variation is normal. Measure the longest needle(s) in the fascicle and record that length in your data sheet.
10. Continue to measure the other nine fascicle lengths. Once you finish measuring one, place it separately to the side. Don't lose these bundles because you can use them as the source of needles for the Needle Damage Assessment protocol.
11. Calculate the average fascicle length and record on your data sheet.
12. Continue working with these 10 fascicles in the Needle Damage Assessment protocol or place them in a small, labeled, zip-loc bag and into the cooler or refrigerator for future use.

NEEDLE RETENTION AND FASCICLE LENGTH DATA SHEET

Tree # _____ School:_____ Town:_____

Tree Quadrant: N S (Circle one)

Date:_____

Student(s):_____

Teacher:_____

NEEDLE RETENTION: 1 2 3 4 (Circle one)

Year of needle development/growth: _____

FASCICLE LENGTH: (Measure ten and average to nearest 0.1 cm)

_____cm

_____cm

_____cm

_____cm

_____cm

_____cm

_____cm

_____cm

_____cm

_____cm

TOTAL _____cm

AVERAGE _____cm

Transfer this information to the Master Data Sheet to be sent to UNH.

NEEDLE RETENTION AND FASCICLE LENGTH

TEACHERS RESOURCE

Introduction

In this protocol, students will be asked to examine pine branch samples in order to determine how many years worth of needles they contain and then to sample at least ten fascicles from the current-year's growth and measure their length in centimeters. These activities move along quickly, especially if you have reviewed needle retention and branch anatomy before students begin on their study samples. Because this is so, it is easy to move directly into the Needle Damage Assessment protocol on the same day.

Guiding Question

What can the needles tell you about the health of your tree?

Materials

- white pine branch morphology diagram
- needle sample bags from the North and South quadrants for all five trees
- Needle Retention and Fascicle Length Data Sheet
- millimeter ruler
- calculator

Background

Each spring, white pines, and other trees in the forest, begin to take up water through their roots. This triggers a process in which the young buds at the tips of a pine branch begin to grow and a new set of needles develops. Each bud contains all the cells of the future needles in their embryonic state. The expansion of the needles occurs as they fill with water and begin to photosynthesize. The buds, therefore, represent the future needle growth for the upcoming growing season. In mature trees, the buds may also hold the reproductive structures: the male and female cones. There are many, many small branches, all with maturing buds, found in the actively growing portion of the tree. Every year, the branches grow a little longer as a new set of needles matures.

The needles found just below the terminal bud are the needles that grew last summer. These are the needles that we will be sampling for the Forest Watch study. We will refer to them as current-year needles. They are now about one year old. One-year-old needles can be distinguished from two-year-old or older needles in several ways:

a. One-year-old needles should be just behind the terminal bud at the end of the branch.
b. One-year-old needles may be lighter or brighter green in color.
c. The bark of the stem that one-year-old needles are attached to may be a lighter brown color than the older stem segments found further down.

All evergreen trees produce new needles every year. Some types, such as the tamarack, lose all of their needles every fall and grow new ones in the spring. Others keep several years worth of needles on their branches at one time. Hemlock, for example, may retain its needles for three to five years while spruces and firs could hold on to their needles for as many as fifteen years. A healthy Norway spruce may keep up to twenty years of needles.

Needles will remain on a branch as long as they can contribute to the overall growth of the tree. The needles are the photosynthetic organs on a conifer tree, and as long as a given age-class of needles remains photosynthetically active, they will be retained on the branch. Once the needles are no longer capable of photosynthetic activity, however, the needles will be cast (lost) from the branch. Air quality factors such as ground-level ozone will accelerate the aging process at the cellular level, resulting in premature loss of photosynthetic activity. Tree species that are sensitive to ozone will exhibit premature needle loss.

The term needle retention refers to the number of years worth of needle growth found on a pine tree branch. A white pine may have from one to four years worth of needles on its branches at the same time. Very healthy white pines may have needles from three or more growing seasons.

Management Suggestions

It is important to note that if samples are collected in the spring, the current-year needles being measured were actually produced last year. If samples are collected in the fall, the current-year needles were produced at the beginning of the summer just past. For example, needles collected in the spring of 1999 were produced in late spring of 1998 but needles collected in the fall of 1999 were produced in late spring of 1999. Either season is acceptable to sample during, just make sure you accurately record the year of the most recent needle growth.

Needle retention and fascicle length are both required protocols in which you will send your data to UNH.

Begin now to train your students to always and immediately return their zip-loc sample bags to the cooler or refrigerator after they have retrieved the material for a particular activity. This is necessary in order to conserve water for the water content study and to prevent any deterioration in the condition of the needles that might later be interpreted as a sign of needle condition in the field. Needles can be kept for quite some time in the sealed bags with a damp paper towel in the refrigerator.

Practice ahead of time looking for bud scale scars and determining needle retention with your class on some pine branches brought in from other trees before you actually sample your white pine trees. Constantly remind your students, each time they begin a protocol with the needles, that they make sure they are using the most recent growth, that from last summer. It is important to note that side shoots on the pine branch will have the same annual growth patterns as the main growing shoot. Make sure that your students select fascicles and needles from these side shoots as well, always choosing only last summers needles.

Students should complete the activity on morphology and needle retention before doing the actual protocol with their needles.

Compare your school's data on needle retention and fascicle length over several years. Describe any noticeable changes.

Identifying Characteristic Conifer Branch Morphology

ACTIVITY

Background

The characteristic shape or morphology of organisms is one way that botanists identify and distinguish between various species. The eastern white pine and other conifers have many similarities that show they share some common genetic characteristics. There are, however, several particular features that distinguish each species from one another.

Some of the features that we can easily focus on to identify and distinguish between the species of conifers are: leaf (needle) length and width, leaf (needle) shape, number of needles in a fascicle and number of years of needle retention on a healthy tree.

A typical white pine will generally show the following traits:

1. Needle width: less than 3 mm or 1/8 in.
2. Needle length: long, thin, soft needle ranging from 75-130 mm long.
3. Five needles found in each fascicle, or bundle.
4. Needle retention: three years or more of needle growth present on a very healthy white pine, two years worth of needles may or may not indicate the beginning of stress, only one year's growth may indicate a stressful environmental impact and reduced photosynthetic capacity in older needles.

Materials

- several branch samples from local conifers including the white pine
- hand lens (optional)
- razor blade
- graph paper
- calculator

Part 1: Identifying White Pine Branch Morphology

Procedure

1. Obtain a piece of a white pine branch from your teacher.
2. Follow along as your teacher helps you examine the white pine branch for several important distinguishing characteristics.

3. Draw and label your white pine branch using the following terms. Describe each structure and what its function is or how it was formed. Refer to the diagram that your teacher has provided you with.

terminal bud	first-year (one year old) needles	two-year-old needles	
fascicle	yearly increment of growth	bud scale scar	third-year branch

4. Examine your branch again carefully. Describe any variations that you see in the following traits:
   needle size color evidence of damage from insects, weather, etc.

5. Trade branch samples with others in your class and observe both the similarities and variations among the white pine branches. What variations do you observe?

6. Remove a bud from the end of a twig. Carefully, using the razor blade, slice the bud in half longitudinally (lengthwise). Examine the inside of the bud. Use a hand lens or stereo microscope if you have one available. Do you see any color? If so, what does this tell you about the bud's content? Draw what you see.

7. What is the function of the terminal bud for the plant?

Part 2: Observing other species of Conifers for their Characteristic Morphology

Procedure

1. Obtain small branches of fir, spruce, hemlock and white pine from your teacher.
2. Observe each species and describe the traits you notice in the table below.

Needle color: Note as green or dark green.
Needle length: Make your measurement in mm.
Needle shape: Draw an individual side view of a needle of each species.

Conifer Type	Needle Color	Needle Length (mm)	Growth Pattern: Single or Group	Needle Shape (draw side view)
Fir (Abies sp.)				
Spruce (Picea sp.)				
Hemlock (Tsuga sp.)				
White Pine (Pinus strobus)				

Other observations

3. List three factors that might influence needle retention on a conifer tree.

CONIFER BRANCH MORPHOLOGY ACTIVITIES

TEACHER RESOURCE

Morphology is the study of the form and structure of an organism. In these activities, students will compare branch morphology among the various species of conifers.

The activities in this section can serve as a great way to learn about needle retention and fascicle measurements before students do the actual protocols with their sample white pine trees. This will allow the students to quickly and efficiently complete the Needle Retention and Fascicle Length protocol and then immediately move into the Needle Damage Assessment protocol.

Guiding Questions

What are the parts of a white pine branch?

How do white pines compare to other conifers in the forest?

Background

The characteristic shape or morphology of organisms is one way that botanists identify and distinguish between various species. The eastern white pine and other conifers have many similarities that show they share some common genetic characteristics. There are, however, several particular features that distinguish each species from one another.

Some of the features that we can easily focus on to identify and distinguish between the species of conifers are: leaf (needle) length and width, leaf (needle) shape, number of needles in a fascicle and number of years of needle retention on a healthy tree.

A typical white pine will generally show the following traits:

1. Needle width: less than 3 mm or 1/8 in.
2. Needle length: long, thin, soft needle typically ranging from 75-130 mm long.
3. Five needles found in each fascicle, or bundle.
4. Needle retention: three years or more of needle growth present on a very healthy white pine, two years worth of needles may or may not indicate the beginning of stress, only one year's growth may indicate a stressful environmental impact and reduced photosynthetic capacity in older needles.

Materials

- white pine and other conifer branch samples
- hand lens (optional)
- razor blade
- graph paper
- calculator

Management Suggestions

Watch the video that explains needle retention, fascicle measurement and needle damage assessment both on your own and with your students.

Make a copy of the pine branch morphology guide for each student to keep and refer to later during the needle retention protocol. Use an overhead copy while you explain how to identify the number of years growth on the pine branch pointing out the bud, current-year growth, bud sale scar, etc. Each student should have a pine branch to hold and identify as you orally describe the parts and functions. They may then go back and draw and label their own samples. Emphasize the fact that such traits as stem and needle length may vary from sample to sample. The more samples you and your students look at the more you will understand and expect this variation.

References

- Campbell, Hyland and Campbell, *Winter Keys to Woody Plants of Maine*, University of Maine Press, Orono, ME, 1978.
- Dirr, *Manual of Woody Landscape Plants*, Stipes Pub. Co., Champaign, IL, 1983.
- Gleason and Cronquist, *Manual of Vascular Plants of Northeastern United States and Adjacent Canada*, Willard Grant Press, Boston, MA, 1963.

Answer Key

Part 1: Identifying White Pine Branch

3. Students should use the pine branch diagram from the overhead or one given to them as a guide. The definitions and functions are obtained from the background information.
4. The most common variations will be in needle length: short, broken needles may be the result of insect damage; needle browning on the tip is dying or dead tissue called tip necrosis from a variety of causes (see needle damage assessment activities); or yellow needle spotting due to air quality or other problems.
7. The bud is the bundled embryonic tissue for next year's growth of twig, cone or flower. The bud should resemble the needles and stem pattern of the mature twig before expansion.

Part 2: Observing other Species of Conifers for their Characteristic Morphology

1. Based on the type of Conifer that you are able to bring to the lab, the following characteristics of needle color, shape and pattern of growth may be seen:

Conifer Type	Needle Color	Needle Shape	Needle Growth	Retention
Fir (Abies)	Green	Flat, blunt tips (friendly)	grow singly, 12-25 mm	as many as 15 years
Spruce (Picea)	Green	Short, Sharp	grow singly, 10-30 mm	as many as 15 years
Hemlock (Tsuga)	Dark Green	Short, Flat	grow singly, 10-15 mm on short stem	3-5 years
Pine (Pinus)	Dark Green	Long, three sided	grow in groups of 5, 75-130 mm	1-3 years

3. Trees do not share the same needle retention characteristics. White pine may only have two years worth of needle growth, while a spruce or fir may have ten or more years worth of needle growth. Some trees lose their needles due to atmospheric pollutants (ozone, sulfur dioxides, aerosols, etc.), others to freeze/thaw cycles, others to insect defoliation, others to fungal, virus or disease causing agents.

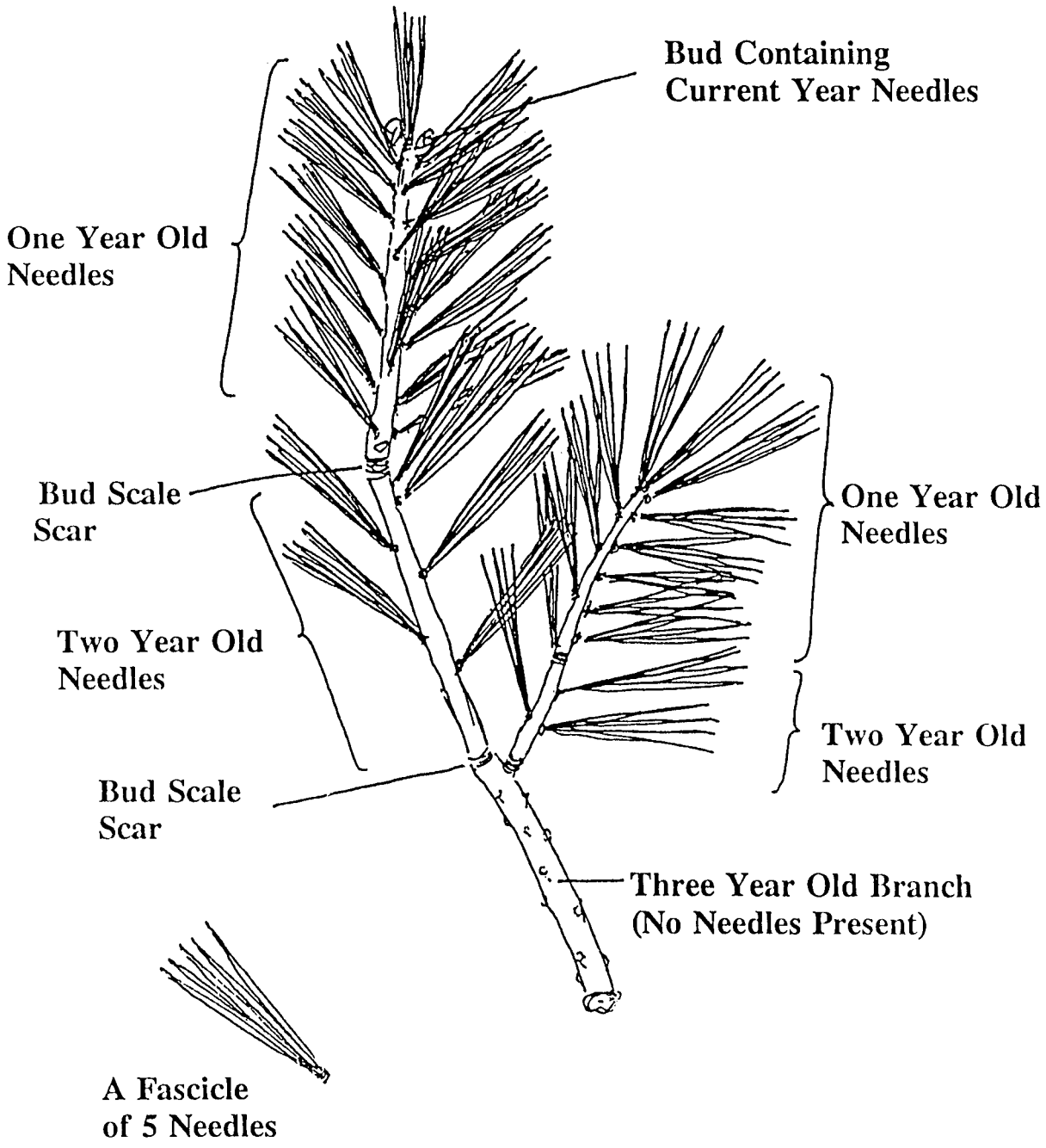
Additional Suggestions

Examine the branch and twig patterns associated with a number of other conifers.

Compare and contrast pine branch and twig patterns with that of deciduous trees.

Compare the fractal patterning of tree branch and leaf growth to that of appendages on animals.

Branch of *Pinus strobus* (White Pine)



NEEDLE DAMAGE ASSESSMENT

BACKGROUND

Guiding Question

How can you determine if a needle is affected by ozone?

Eastern white pine trees affected by ozone pollution may display two different symptoms of damage on their needles: ozone injury and/or chlorotic dwarf. Ozone injury includes damage to areas on the needles in the form of chlorotic mottle and tip necrosis. If ozone exposure occurs during the early growth phase of the needles, the needles may not grow as long as they should. The resulting stunted condition is known as chlorotic dwarf.

Ozone affects needles because it enters a needle through the stomates. Once inside, it begins to damage the mesophyll cells next to the stomate. Under severe conditions, the damage can spread to additional cells inside the needle, possibly leading to needle death. The first signs of ozone damage may be tiny areas of yellowing on the needles where chlorophyll is no longer functioning properly and the characteristic green pigmentation is no longer present.

In this exercise, you will learn how to identify areas on a needle that indicate ozone damage and record whether or not the injury is present on the needles. You will be measuring and recording the length of randomly chosen current-year pine needles. You will then be looking for two ozone symptoms: chlorotic mottle and tip necrosis. We are focusing on these two symptoms because we have sampled mature needles that may have been affected by ozone damage not only in the developmental stages, but throughout the summer months when ozone episodes are most likely to occur. Scientists have determined that the occurrence of both chlorotic mottle and tip necrosis on the same needle, in the absence of other causes, is an indication of ozone damage.

In addition to looking for specific ozone symptoms, you will measure the cumulative total needle damage, which will affect the spectral properties of the needles. Because ozone enters needles through the stomates, we will limit our observation of ozone damage symptoms to the stomatal side of the needle. Measurement of total needle damage should be taken on the same side that was studied for chlorotic mottle. In another exercise, you may actually look inside needles by making cross-sections of the needles. Then, using a microscope, you will try to identify cells that may be damaged by ozone pollution, especially those adjacent to stomates.

Description of ozone symptoms that will be measured in this activity:

Chlorotic mottle- This type of damage occurs as yellow regions on a needle that have indistinct rather than sharp edges. Because cells are being affected by ozone gas on the inside of the needle, damage may occur unevenly as more and more cells are injured. This means the shape and size of the mottle may vary.

Tip necrosis- This type of damage occurs when the tip of the needle turns brown as cell tissue dies from exposure to ozone or other air pollutants. Tip death of needles can also occur if the tree is experiencing environmental stresses such as extreme drought or winter wind burn. These conditions may cause a die-back of the needle as it attempts to conserve water and/or other resources. Tip necrosis can vary in length.

Another possible symptom of ozone pollution which you may observe, but <u>will not</u> be measured in this activity:

Chlorotic dwarf- This disease involves the influence of ozone pollution on the development and full maturation of needles. In the early spring, if the emerging needles are exposed to ozone pollution, their natural bright green color may become spotted with chlorotic mottling. This process slows down their growth. Other environmental factors, such as drought, may further weaken the current-year needles and cause tip necrosis. These damaged needles never reach their normal length.

Discussion Questions

1. How does ozone enter white pine needles and where does damage first occur?

2. What are three symptoms of ozone damage?

3. What two symptoms occurring on the same needle, in the absence of other causes, are indicative of ozone damage?

4. What two symptoms will you be measuring in this study?

5. When are needles most susceptible to ozone damage?

NEEDLE DAMAGE ASSESSMENT

PROTOCOL

How can you identify ozone damage on white pine needles?

Materials

- white pine needle tufts from the north or south quadrant
- hand lens and/or dissecting microscope
- metric ruler
- reference materials
- Datesheet

Procedure

1. Select either the north or south sample bag from the cooler or refrigerator for your tree. Remove one branch tip with several needle fascicles attached (a "tuft" of needles). Immediately close the bag and return it to the cooler or refrigerator.
2. Randomly select 30 fascicles from the current-year needles on your tuft. You may need to use two or more tufts in order to get the 30 needles. Remove 1 needle from each of those fascicles. These will be your sample needles.
3. Measure and record the length in mm of each needle on the Needle Length and Injury Data Sheet.
4. Using a hand lens or dissecting microscope, observe the needle for signs of tip necrosis. If you observe tip necrosis on the needle, record a yes on your data sheet. In some cases, the tip of a needle might be broken off and missing. Make a note of this on your data sheet.
5. Using a hand lens or dissecting microscope, observe the stomatal side of the needle for signs of chlorotic mottle. If you observe one or more areas of chlorotic mottle on a stomatal side, record a yes on the line in your data sheet.
6. Measure and record the total length in mm of any damage observed on the needle using the same side that you observed for chlorotic mottle. You do not need to distinguish between the damage symptoms, simply add together the lengths of all damage types you observe.
7. Transfer all data to the Master Needle Length And Injury Data Sheet**.** Use one sheet for each quadrant. You should have a minimum data set of 30 needles from each quadrant. This will provide enough data for further statistical analysis of your findings, if you wish to do that.

REMEMBER: Observe and record chlorotic mottle on the stomatal side of the needle.

NEEDLE LENGTH AND INJURY DATA SHEET

Students/Class:_____Date: _____

School:_____ Teacher:_____ Town:_____

Tree #:_____ Quadrant: N S (circle one)

	Needle length **(in mm)**	Tip Necrosis **(yes or no)**	Chlorotic Mottle **(yes or no)**	Total Damage **(in mm)**	% Damage (in mm) **tot. damage (mm) / tot.length (mm)**
1.					
2.					
3.					
4.					
5.					
6.					
7.					
8.					
9.					
10.					
11.					
12.					
13.					
14.					
15.					
16.					
17.					
18.					
19.					
20.					
21.					
22.					
23.					
24.					
25.					
26.					
27.					
28.					
29.					
30.					

mean needle length _____mm, mean length total damage_____mm,
mean % total damage_____, % needles with tip necrosis_____, % needles with chlorotic mottle_____, and % needles with both symptoms together_____.

Send this data sheet to the UNH. You need to send data from the N and S quadrants for all five trees.

Analysis

1. Obtain the master data sheet that contains the data for all the needles in the same quadrant (North or South) for the tree with which you are working. You should have data for at least 30 needles:

   a. What is the total # of needles in your new sample? _____

   b. Calculate the average needle length for the needles in your new sample: _____mm

   c. Calculate the average length of total damage for the needles in your new sample: _____mm

   d. Calculate the average percent total damage for the needles in your new sample: _____mm

   e. How many needles have at least one occurrence of chlorotic mottle? _____

      Calculate the % of needles that have chlorotic mottle: _____

   f. How many needles have tip necrosis? _____
      Calculate the % of needles that have tip necrosis: _____

   g. How many needles have both chlorotic mottle and tip necrosis?_____

      Calculate the % of needles that have both symptoms: _____

2. Order your data by rearranging it in a table that lists needle length from longest to shortest. Alternately: (Use a stem and leaf diagram to order the data for needle length measurement).

3. Locate the median and describe the range for the needle length data.

4. Look for patterns:

   a. Construct a frequency table in which you sort information into four categories:
   the number of needles without any ozone symptoms, the number of needles with chlorotic mottle only, the number of needles with tip necrosis only and the number of needles that show both ozone symptoms.

   b. Construct a bar graph of your data comparing the total number of needles, the number of needles with no symptoms, the number of needles with chlorotic mottle only, the number of needles with tip necrosis only, and the number of needles with both symptoms occurring together.

Extra Credit

Construct a box plot using the needle length data. Use the box plot to describe the amount of variation that exists in this quadrant.

Interpretation

Use the tables, graphs, and plots you have constructed to answer the following questions.

1. From your data, does there appear to be a relationship between needle length and occurrence of chlorotic mottle? If so, describe the relationship you observe. Would you normally expect to see a relationship between needle length and the occurrence of chlorotic mottle? Why or why not? Speculate as to why a data set might show an apparent relationship between two measurements when in fact there really isn't one.

2. From your data, does there appear to be a relationship between the occurrence of chlorotic mottle and the occurrence of tip necrosis for the needles in your quadrant? If so, what is that relationship?

3. Obtain the data your classmate has analyzed for the opposite quadrant of your tree. Compare the occurrence of tip necrosis, occurrence of chlorotic mottle and occurrence of both symptoms between the quadrants. What similarities or differences do you observe?

4. Compare mean needle length, median needle length and the amount of variation in each set between quadrants.

5. If possible, obtain information from your classmate who has made a box plot for the opposite quadrant of your tree and compare it to the box plot you may have made. What similarities and differences do you observe?

6. Refer to the data for average percent total damage. Does this information indicate to you that your needles have a lot of damage? Compare this information with the information about tip necrosis and chlorotic mottle occurring on both needles. Is the average percent total damage also a good measure of damage from ozone? Explain.

Communicating Your Analysis and Interpretations

Scientific research has shown that white pine needles showing the injury symptoms chlorotic mottle and tip necrosis, both occurring on the same needle, may in fact have been exposed to ozone pollution. Part of our research mission is to get more information about this association. This information alone, though, is not enough to determine if ozone is damaging our trees and forests.

Look back carefully at your data, analysis and interpretation. In a short summary paragraph, make a statement about the needles on your tree and their possible exposure to ozone pollution. What other information about the needles, tree or environment would help you determine if your tree was damaged by ozone?

Hint: If available at this time, check your results for water content, chlorophyll analysis, cross sections, VIRIS, and other observations to help you complete this section.

NEEDLE ASSESSMENT

TEACHER RESOURCE

Introduction

Using observation techniques, students will observe various forms of external needle damage and their possible causes. Using observation and measurement techniques, students will assess possible ozone injury to needles.

Guiding Questions

How can you observe symptoms and needles for environmental damage?

What factors in the environment affect white pine needles?

How can you determine if a needle is affected by ozone?

Materials

- white pine needles
- hand lenses and/or dissecting microscopes
- metric rulers
- reference materials

Background

The activity "Can You Observe Injuries to White Pine Needles?" should be completed before the needle damage protocol.

The introductory needle damage activity introduces students to the various ways that white pine needles may be damaged by forces in their environment. Students list and discuss possible causes for the changes they observe on the needles.

The protocol serves to focus students specifically on ozone damage symptoms and how it affects needles. Students measure and analyze their results and then make a prediction about the health of their needles relative to ozone pollution. Students are asked to provide examples of other information that would help them assess the health of their tree with respect to ozone pollution.

Additional information on typical needle damage that students may observe:

Needle damage can occur by many agents both biotic and abiotic. Chlorosis is a general term that refers to a yellowing of the needle. It can be caused by such biotic agents as fungi, bacteria, viruses and insects, as well as by air pollutants and other abiotic stresses. Sometimes, it is difficult to distinguish between ozone-caused chlorosis and chlorosis caused by other sources. For example, chlorosis caused by sulfur dioxide pollution is virtually indistinguishable from ozone mottling on a white pine. Recall, however, that in this study we are assessing several signs of ozone impairment including such measures as the spectral

characteristics of the needles as well as external and internal anatomical damage to tissues. Ideas for how to distinguish ozone chlorosis from other forms are discussed in the section below.

Since ozone enters the needle through the stomates and first damages the nearby cells, observations of chlorotic mottle on the non-stomatal side of the white pine needle cannot be attributed unequivocally to ozone damage. Other situations where pine needles could be damaged include road salt spray on the needles of trees near roads and highways, gypsy moth caterpillar infestations (rarely), tip necrosis from frost damage, desiccation due to winter wind exposure, animals or insects chewing on needles, dwarfing of needles caused by drought periods during development, among many other possibilities. Observation of meteorological conditions during the period of needle development (i.e., early summer drought) or unusual winter weather that could damage mature needles could help characterize the nature of the needle injury.

Additional information on the effects of ozone on white pine:

Sensitive trees can incur ozone injury in situations where the summer daytime average atmospheric concentrations of the gas often exceed 0.05 ppm. Damage will also take place when the maximum hourly average often exceeds 0.1 ppm. Sensitivity and reaction to ozone varies at low concentrations, but at concentrations of 0.1 ppm or higher, all effects of ozone on plants are negative.

Ozone gas is highly reactive and it breaks down during reactions within the leaf or needle. Since no detectable byproducts are produced, ozone injury to the cells and needle surface are the diagnostic tools used to detect ozone as the agent of damage. Ozone causes the membranes of cells and cell parts to collapse and lose function. Often, several cells will collapse in a cluster. In pine needles, mesophyll cells near the stomata are often the first to be impaired. The two most characteristic symptoms of ozone damage are chlorotic mottle and tip necrosis occurring on mature or semi-mature needles. We will be focusing on the occurrence of both these symptoms on the mature needles sampled in our study.

Chlorotic mottle may develop along nearly the entire length of mature needles. It appears as small yellow blotches with diffuse margins. Look for mottling along one of the stomatal sides of the needle.

The early stages of tip necrosis can occur when a summer ozone episode takes place after needle emergence during the intermediate stages of development. The most sensitive area is the semi-mature tissue found in-between the base of the needle and the more mature tip tissue above. The region around the stomates will initially show tiny bleached or pink spots that may eventually develop into pinkish-yellow bands. As more cells die, the band may turn brown and progress toward the needle tip eventually causing tip necrosis.

Minor injuries to needles can be intensified during the winter by desiccation or freeze/thaw episodes. Other flecks, spots and injuries can be distinguished from ozone injury if they occur on the non-stomatal needle surface. Observation of affected areas through thin-sections and microscopic analysis of cell damage will help to further confirm ozone damage.

Plant leaf tissues can be damaged when ozone in the atmosphere diffuses into the leaf through the stomates. The ozone collects in the substomatal cavities and actively degrades cell membranes. This reduces the cell’s ability to carry out photosynthesis. Interestingly, however, researchers have found that when ozone reaches a high level of concentration in the atmosphere, the stomates of a leaf may close.

This mechanism could act to reduce the potential damage to cell tissue. The level of ozone exposure and the length of exposure may vary from species to species and is presently an area of on-going research.

Bibliography for this section:

Sinclair, W. A. et al. 1987. *Diseases of Trees and Shrubs*, Cornell University Press,Ithaca, NY.

Management Suggestions

Observation activity:

Remind students to bring in samples of white pine branches the day before the observation activity and have extras on hand. Have available reference materials including drawings, video, written references and actual samples (if possible) to help illustrate various pine needle injuries. Discuss how insects and disease can affect the forest industry and how the industry and private individuals try to combat these problems (pesticides, genetic engineering, etc.). Allow one class period for this activity.

Measurement activity:

Students may work in pairs measuring ten needles at a time using the data worksheet. Then, following the procedure, should combine their data with other students', or other work they have done, for the same quadrant to get a total of 30 needles per side. This information can be used for the discussion questions and should be transferred to the Master Data Sheet and sent to UNH. Instead of copying all the data again onto the Master Data Sheet, you may make photocopies of your own Needle Length and Injury Data Sheets each with 30 needles for each tree, north and south quadrants, and send them to UNH.

Continually remind students how important it is that care and accuracy be used while they measure needle length and total length of needle damage. Careful observation of the occurrence of chlorotic mottle and tip necrosis is important too. Students should be looking for chlorotic mottle on the stomatal sides of the needles. It may be helpful to hold the needle in place under the microscope using a pair of tweezers, or try to balance the needle on a crumpled piece of tissue. Circulate around the room and be available to answer questions and clarify identification of ozone symptoms. Students and teachers often have a hard time identifying chlorotic mottle. The more examples you and your students look at the more familiar you will become with identifying ozone injury.

Examples of data manipulation techniques suggested in the analysis portion of the measurement activity can be found in the Math Activities section of this guide. Student will complete analysis questions on a separate page or lab sheet.

Additional Suggestions

Look for certain features in the stem and leaf plot to help determine how the data is distributed:

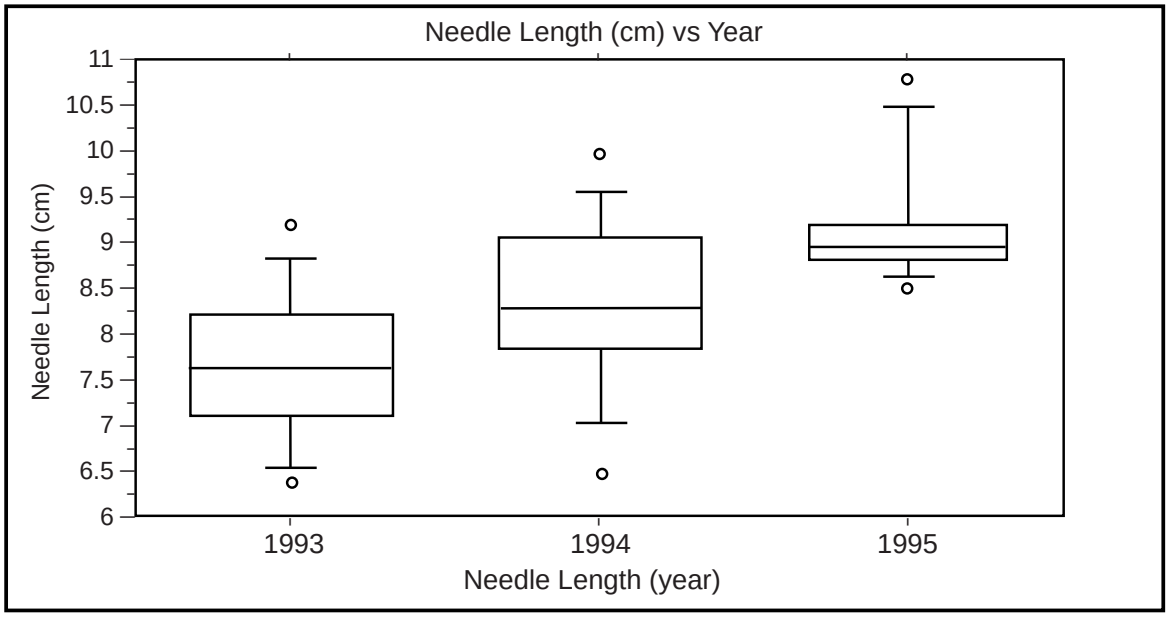
1. normal vs. skewed shape of the curve
2. range of data
3. outliers
4. clusters

Box Plots

Use the descriptive information to construct box plots of the data. Several box plots can be constructed to show how the data change with time. Indicate outliers using an O on the graph.

Sample data:

	low outlier	10th	25th	median	75th	90th	high outlier
1993	6.4	6.6	7.1	7.6	8.2	8.9	9.2
1994	6.5	7.1	7.8	8.3	9.1	9.6	10.0
1995	8.5	8.7	8.8	9.0	9.2	10.5	10.8



Needle measurement data can be compared with other measures in a variety of statistical tests. Try comparing needle length to wet weight/dry weight results and some of the spectral data in scatter plots or, for the advanced student, correlation and regression analyses.

Use data from previous years to perform ANOVA (Analysis of Variance) tests for needle length between years for one school or between schools for different areas. There are many possibilities.

Time to complete activities:

At least 1 class period to practice and become familiar with symptoms. At least two or three class periods to gather, share and analyze data. Some parts may be assigned as homework.

Answer Key

1. From your data, does there appear to be a relationship between needle length and occurrence of chlorotic mottle? If so, describe the relationship you observe. Would you normally expect to see a relationship between needle length and the occurrence of chlorotic mottle? Why or why not? Speculate as to why a data set might show an apparent relationship between two measurements when in fact there really isn't one?

   There is no evidence that longer or shorter needles will have more or less chlorotic mottle on them due to length of needle alone. It is possible that a pattern could arise and so it is important to remind students that some data may suggest a relationship that really does not exist. This can occur simply by chance in sampling. This underscores the importance of gathering large enough sample sizes in order to minimize chance anomalies in the sample population.

2. From your data, does there appear to be a relationship between the occurrence of chlorotic mottle and the occurrence of tip necrosis for the needles in your quadrant? If so, what is that relationship?

   Answers will vary depending on the particular data set. The symptoms chlorotic mottle and tip necrosis can occur and frequently do occur one without the other on a needle. If students notice that a large percentage of the needles sampled have both symptoms on them, then that may indicate exposure to ozone pollution.

3. Obtain the data your classmate has analyzed for the opposite quadrant of your tree. Compare the occurrence of tip necrosis, occurrence of chlorotic mottle and occurrence of both symptoms between the quadrants. What similarities and differences do you observe?

   Again, as stated in question #2, answers will vary. Differences between quadrants may lead the students to speculate as to what factors in the environment might produce such differences. Do the differences occur by chance or are the differences statistically significant? How would we go about determining statistical differences in the data set? (These can be thought questions or, for advanced students, a further inquiry).

4. Compare mean needle length, median needle length and the amount of variation in each set between quadrants.

   Answers will vary. Are the differences large or small? Emphasize that without information about the variation in the data set, we really cannot say with confidence if the quadrants are different from one another. Is there an overlap in the variation or are they quite different from one another. How do one or two very long or one or two very short needles skew the mean of the data set? Is the median also a good measure of the mean in this data set? Why or why not?

5. If possible, obtain information from your classmate who has made a box plot for the opposite quadrant of your tree and compare it to the box plot you may have made. Do you observe any similarities or differences?

   See answer to # 4. This provides a visual representation of variation. Review the box plot example in the teacher resources section that compares the needle length for three years in a row. In cases where the means of the data fall within the boxes of the other sample sets, the differences in the data sets may be due to chance variation in the sample itself rather than real differences between the quadrant populations.

6. Refer to the data for average percent total damage. Does this information indicate to you that your needles have a lot of damage? Compare this information with the information about tip necrosis and chlorotic mottle occurring on both needles. Is the average percent total damage also a good measure of damage from ozone? Explain.

   Students will have to determine what "a lot" of damage means to them. We don't know how much "damage" a needle can sustain and still be able to function at a healthful level. This kind of knowledge may only come from many years of observation and experience. Students can, however, speculate about the relative effects of various amounts of damage and can relate total percent damage to the observed amount of chlorotic mottle/tip necrosis on the needles. Needles with a high percentage of total damage, but few instances of both chlorotic mottle and tip necrosis on the same needle, are probably less likely to be suffering from ozone pollution than from some other environmental factor.

CAN YOU OBSERVE INJURIES TO WHITE PINE NEEDLES?

ACTIVITY

What factors in the environment affect white pine needles?

Eastern white pine trees can be negatively affected by a number of biotic and abiotic influences. These may include animal, insect, fungal and bacterial damage as well as the condition of such factors as the soil and atmosphere that the tree grows in. One particular atmospheric condition that affects white pine is the presence of the pollutant ozone, which when present in the atmosphere at particular levels and for certain periods of time can injure the tree, particularly the needles and how they function.

In this activity, you will observe needles externally for color changes and evidence of injury. In the next activity you will look more closely at specific symptoms of ozone damage and attempt to measure the amount of damage on a needle. You may want to bring in samples of white pine needle tufts from home to first familiarize yourself with typical needles and the various injuries they may incur before you assess your sample trees.

Materials

- white pine needle tufts
- hand lens and/or dissecting microscope
- reference materials

Procedure

1. One of the objectives of this study is to assess injury on needles that grew and developed last summer. First however, you should look at your entire tuft of needles and make some observations. Make a list of what you observe and compare your list with other students in your class.
   a. Did you observe any color variation among the needles? If so, describe them.
   b. Did you observe any evidence that insects or other animals may have affected the needles in your tuft? Describe what you saw and speculate as to what might have caused those injuries.
2. Remove a few needle fascicles from different places in the tuft. Is there variation among the fascicles? How are the needles within a fascicle similar or different from one another? What might cause needles to vary in length between and within the fascicles?
3. By now you may have noticed a pattern of small spots that run along one side of your needle. Make a hypothesis about the function of these structures. Compare your hypothesis with others.
4. As a class, list and discuss what you have observed about the needles you studied. You will probably find that there are many possible factors that can change the structure and function of white pine needles.

Analysis and Interpretation

1. One of the main functions of a needle is to convert radiant energy from the sun into chemical energy that the plant can use to grow and develop. The process involved is called photosynthesis. The stomates of a needle take in atmospheric gases so the cells of the needle can then use some of those gases in the process of photosynthesis. If this process cannot take place in needles, then the needles cannot make the food the tree needs and the tree may become weakened and even die.
   a. How do you know that photosynthesis has taken place in the needles you observed? Can you tell by the color?
   b. Refer to your list of observations related to needle color. What may be happening in the areas of a needle that do not show the characteristic green pigmentation associated with photosynthesis? Speculate as to what might happen to a tree if many needles were yellow or brown.
   c. What environmental factors might cause a needle to change color from green to yellow or brown?
   d. Besides the gases that are used in photosynthesis, what other gases might be taken in by the stomates?
2. Loss of pigments in needles is not the only way that they may lose their ability to function properly. Discuss with your class and teacher various biotic and abiotic factors in your local environment that also affect the way white pine needles and trees may grow and develop.

Communicating Your Findings

Write a short statement about what you learned from this activity. What is the relationship between the ability of a needle to function properly and the environment that the needle grows in?

DETERMINING WATER CONTENT OF WHITE PINE NEEDLES

BACKGROUND

Guiding Questions

How do plants utilize water?

How can we determine the percent water content of our needles at the time of collection?

There are many factors that influence the development of a needle. These may include water availability, insect infestation, disease and air pollution. In this activity, you will examine how the amount of water in needles may vary and possibly affect development. You will determine the amount of water found in the needles at the time of collection. The percent water content of the needles at the time of collection may be a reflection of the state of health of the tree.

Water is important for the process of photosynthesis in a plant. Water also affects the needle as it elongates and matures. One very important factor affecting the amount of water in the needles you collect is the time of day that the collection is made. Other factors are related to soil moisture and uptake of water by the tree. These may include the amount of water present in the soil, the level of the water table, depth of root development and the absorption characteristics of the root hairs. Within the tree itself, such factors as the condition of the vascular tissue in the stems and needles, the volume of air space within the needle and the number and function of stomates on a needle all influence the way water may be used by the plant. Lack of or severe loss of water in the plant could change the way the plant grows and might limit the amount of photosynthesis. Without the water that a plant needs to grow and develop, it may weaken and eventually die.

Discussion Questions

1. What factors might affect the development of a pine needle?

2. Why is the amount of available water important to the overall health of a tree?

3. Consider the water table and how its level can change during different parts of the year. Under what conditions could water be present in the ground and yet not be available to a tree? How does the water table become replenished? List at least two ways?

4. How does the amount of water in a healthy white pine needle compare to the amount of water in your body?

DETERMINING THE WATER CONTENT OF WHITE PINE NEEDLES

PROTOCOL

How can you find the amount of water contained in pine needles?

Materials

- needle samples from the north and south quadrants of your tree
- Celsius thermometer (Fahrenheit thermometer may be substituted)
- balance (should measure to at least .01 grams)
- Wet/Dry Weight Data Sheet
- drying oven
- stapler
- paper bags (lunch bag size)

Procedure

1. Obtain one of the zip-loc bags that contains needles for your assigned tree from the cooler or refrigerator. Record the tree # and quadrant information on one of the brown paper bags.
2. Open your zip-loc sample bag and remove a small portion of needles from the current-year's growth. Close the bag again and return it to the cooler. Using a paper towel, blot off any excess water that may be visible on the needles.
3. Accurately mass the current-year needles and record on the section of the data sheet called **Mass of Fresh Needles.** You should have between 3 and 5 grams of needles. Discard any material from the bud if it has already begun to elongate.
4. Carefully place all the needles inside the dried paper bag and close by folding the top of the bag two or three times. Staple the bag closed.
5. Carefully mass the bag and record this mass on the section of the data sheet called **Mass of Bag with Needles.**
6. Place the paper bag in the dehydration oven (set to 50° Celsius) for 3 - 5 days.
7. After drying, remove the paper bag from the oven and accurately mass the bag again. Do not remove the staples or needles from the bag. Repeat this measure for several days, if necessary, until the mass of the bag and needles stabilizes. This will ensure that all the water has been evaporated from the needles. When you are sure the needles are completely dry, record the lowest mass on the section of the data sheet called **Oven-dry Mass of Bag with Needles**.

Note: It is important to mass this oven-dry sample immediately after removal from the drying oven. As soon as the sample is outside the oven, both the sample and the bag begin to remove water vapor from the air.

WET /DRY WEIGHT DATA SHEET

Students/Class:_____ Date: _____

School:_____ Teacher:_____ Town:_____

Tree #'s:_____ Site Coordinates (long. and lat.):_____

RECORD YOUR DATA BELOW (to .01 grams)

		North	South
A. Mass of the Fresh Needles		_____	_____
B. Mass of Bag with Needles:		_____	_____
C. Oven-dry Mass of Bag with Needles:		_____	_____
Continue daily measurement until mass stabilizes**.** Record final mass for C in the space above. Continue with days 5 and on if necessary.	Day 1	_____	_____
	Day 2	_____	_____
	Day 3	_____	_____
	Day 4	_____	_____

1. Determine the **mass** of water in the fresh needles. Show how you do this and record your answer below.

	North	South
Mass of water in the fresh needles	_____	_____

2. Determine the **percentage** of water in the fresh needles. Show how you do this and record your answer below.

	North	South
% water content in the fresh needles	_____	_____

3. Your class may have completed more than one set (trials) of data for your samples. Obtain this information and use it to calculate the average % water content again for the quadrant that you are working on.
4. Record the data from all five trees on the Class Wet/Dry Weight Data Sheet.

Analysis and Interpretation

1. List 2 ways in which the needles that you massed in **B** differ from the needles that you massed in **C**.

2. What substance is in the needles that you massed in **B** that is not in the needles that you massed in **C**?

3. How is water important in plants?

4. How is water important in the photosynthetic process?

5. Use the class data sheet to construct a clustered bar graph of the % water content for the north and south quadrants for all five trees. You may want to use colored pencils to enhance your graph.

7. From your bar graph, what differences do you observe between % water content of the needles from all five trees? Is there any evidence that any of the trees appear relatively unhealthy with respect to water content of the needles? Explain.

8. What factors might account for variation in the amount of water found in the needles?

Communicating Your Results

Refer to the results your class has obtained about the water content of the needles from your trees. How might winter snowfall and spring precipitation be related to % water content of the needles? What other factors might affect the amount of water found in needles? How might water content ultimately affect the overall health of a tree?

CLASS WET/DRY WEIGHT DATA SHEET

Students/Class:_____ Date: _____

School:_____ Teacher:_____ Town:_____

Tree #'s:_____ Site Coordinates (long. and lat.):_____

Collect each group's data and record in the space below

	% Water Content	
	North	**South**
Tree # _____	_____	_____
Tree # _____	_____	_____
Tree # _____	_____	_____
Tree # _____	_____	_____
Tree # _____	_____	_____

Transfer this information to the Master Data Sheet to be sent to UNH.

PERCENT WATER CONTENT

TEACHER RESOURCE

Introduction

In this activity, we will be calculating the percent water content in the needles that emerged and developed last spring. We will mass 3 - 5 grams of these current-year needles from each of your white pine samples. The needles will then be placed in dry paper bags, massed again and stored in a drying oven at about 50 degrees Celsius for 3 - 5 days. The final dry mass will be determined and then used to calculate the amount of water that was originally present in the fresh samples. Percent water content of the needles can then be calculated. Remember, the % water content that is being calculated indicates only the amount of water in the needles at the time of collection. Overall needle length, however, may be related to availability of water at the time of elongation. Students will be asked to speculate on this.

Guiding Questions

How do plants utilize water?

How can we determine the percent water content of our needles at the time of collection?

Materials

- needle samples from north and south quadrants
- drying oven
- celsius thermometer (Fahrenheit thermometer may be substituted)
- balance (should measure to at least .01 grams)
- paper bags (lunch bag size)
- stapler
- data sheet
- plastic zip-loc bags will be needed if the oven-dry samples have to be moved from the oven to another room for massing. (see explanation under Management Suggestions)

Background

In order for photosynthesis to take place, all reactants must be available in sufficient quantity. Water is a vital reactant in photosynthesis and its quantity in the needles is determined by the amount available in the soil, the absorption characteristics of the root hairs, the quality and relative quantity of the vascular tissue (xylem) in the stems and needle petioles, the volume of air space within the needle, and the quantity and condition of the stomatal apparatus in the needle at the time of collection. If any of these conditions is abnormal, the plant may be under severe water loss stress. This stress may result in damage or even death of the plant. Needles under stress may show discoloration, stunted growth and/or loss.

Management Suggestions

1. Paper bags must be oven dried at 50°C for at least 4 hours before completing this activity. This removes water from the bag due to humidity in the air.
2. Students will be using a balance to obtain the mass of the current-year needles before and after drying. Explain to your students that a scale is used to measure weight that takes into account the force due to gravity. A balance is used to measure the mass of an object relative to a known mass. In using a scale you weigh the object, whereas in using a balance you mass the object.
3. Student groups may be assigned to complete the testing protocol for one white pine tree. For example, one group may be assigned to assess percent water content for tree # 1 while another group assesses percent water content for tree #2 and so on. For each tree and for each quadrant, at least one duplicate trial should be run. This means that from each white pine sample, two 3 - 5 gram current-year needle samples from the north quadrant and two from the south quadrant will be assessed separately. This will help students check their accuracy.
4. The initial mass of current-year needles should be determined <u>as soon as possible</u> after collection. Samples must be kept moist and sealed in zip-loc bags to avoid water loss. The second measurement of mass (the dry mass) will be taken 3 - 5 days after the drying process has been completed.
5. At the end of the 3 - 5 days of drying, it is important to mass your oven-dry sample immediately after removal from the drying oven. The longer the sample is outside the oven, the more moisture both the sample and the bag collect from the air. In some cases, your drying oven may be in a different classroom or area from where you are working. If you have to transfer the bags from one place to another, place the paper bag in a zip-loc bag, remove as much air from the zip-loc bag as possible and seal the bag tightly. Immediately take them to the balance, remove one bag at a time from the plastic bag and mass as instructed.

Note: For middle school students, this exercise provides a good opportunity for work with the mathematics teacher on calculating percents.

<u>Beginning the discussion</u>:

Ask your students to consider the following ideas and questions. Why is it important to drink lots of fluids when you are exercising? (Avoid dehydration. If we become dehydrated, our bodies are stressed and we can become sick.). We all know that water is a major substance in the healthy human body. Approximately how much water might that be? (About three-fourths or 75%)

Hold up a sample of pine needles:

How much water do you think is in this sample of pine needles?

If the percent water content of pine needles on a tree becomes significantly less, how might this affect the tree? (It may cause stress in the tree resulting in unhealthy conditions.)

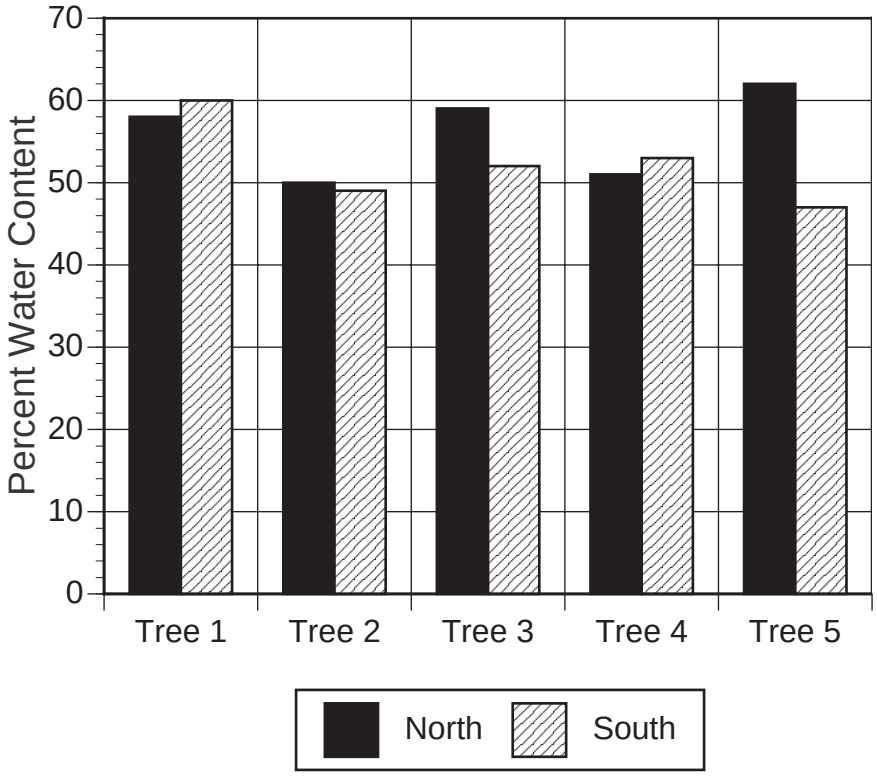
Do you think that it is possible to measure the amount of water in a sample of white pine needles? How could we measure the amount of water in these needles? Brainstorm some ideas.

Additional Suggestions

1. Compare the results from all 5 trees by constructing a bar graph of the data from each tree comparing the N and S quadrants.

Sample data set

	North	South
Tree # 1	58	60
Tree # 2	50	49
Tree # 3	59	52
Tree # 4	51	53
Tree # 5	62	47



For further study:

Assemble a data set for your school for the past 3 or more years (if you have that data available). Obtain the historical precipitation data for your area for each of those years and compare.

Compare needle length and percent water content for the needles.

Include lab activities on evapotranspiration, diffusion/osmosis, and turgor pressure in guard cells of plants such as *elodea* or geraniums.

Answer Key

Determine the **mass** of water in the fresh needles. Show how you do this. Record your answer below.

(Mass of bag with needles) - (Oven-dry mass of bag with needles) = original mass of water. Students must report answers in grams.

Determine the **percentage** of water in the fresh needles. Show how you do this. Record your answer below.

(Mass of water in fresh needles) / (Mass of the fresh needles) X 100 = % water content

1. List two ways in which the needles that you massed in **B** differ from the needles that you massed in **C**?

   Some possibilities are: B has a greater mass, C was dried in the oven for x number of days, and C has lost water because it was dried.

2. What substance is in the needles that you massed in **B** that is not in the needles that you massed in **C**?

   Water

3. How is water important to plants?

   Water is important to plants for many reasons:

   - *cell structure and elongation*
   - *movement of materials across cell membranes*
   - *evapotranspiration - plant's circulation*
   - *osmotic pressure*
   - *a chemical reactant in a hydrolysis reaction*
   - *turgor pressure (wilting, operation of the guard cells around the stomates, for feeding in Venus fly trap)*
   - *seed germination (expansion of the cotyledons and emergence of the embryo)*
   - *reproduction (a substance found in nectar for attracting insects)*
   - *photosynthesis*

4. How is water important in the photosynthetic process?

   Water is one of the chemical reactants in photosynthesis, the process by which plants synthesize their own food (glucose)

$$6CO_2 + 6H_2O \xrightarrow[\text{(light energy)}]{\text{(chlorophyll)}} C_6H_{12}O_6 + 6O_2$$

glucose

5. Use the class data sheet to construct a clustered bar graph of the % water content for the north and south quadrants for all five trees. You may want to use colored pencils to enhance your graph.

   Graphs will vary.

6. From your bar graph, what differences do you observe between % water content of the needles from all five trees? Is there any evidence that any of the trees appear relatively unhealthy with respect to water content of the needles? Explain.

   Answers will vary.

7. What factors might account for variation in the amount of water found in the needles?

   Examples might include: winter, spring, or summer drought, disease, insect infestation, water loss due to plasmolysis caused by ozone or other air pollution, desiccation due to wind, exposure to sunlight causing high rates of evapotranspiration, any of these factors differing between north and south quadrants.

Communicating Your Results

Refer to the results your class has obtained about the water content of the needles from your trees. How might winter snowfall and spring precipitation be related to % water content of the needles? What other factors might affect the amount of water found in needles? How might water content ultimately affect the overall health of a tree?

Statement now includes all trees. Wet vs. dry winter/spring could affect availability of water at needle expansion and therefore affect the potential for the needle to grow. As the water levels drop, photosynthesis and other chemical reactions in the needles may be negatively affected. This could in turn weaken the tree over time and ultimately lead to death.

NEEDLE ANATOMY

C

BACKGROUND

Guiding Questions

What does a normal needle look like under a microscope?

What do chlorotic mottle and tip necrosis look like under a microscope?

What cellular changes result in chlorotic mottle?

In order to understand how tropospheric ozone, and/or other stress factors, may damage the cells of a needle, it is important to become familiar with the structure and function of the major cell and tissue types found in a healthy white pine needle. As you become familiar with the appearance of cells in a healthy needle, you will be better able to spot symptoms of damage in needles that may be due to ozone pollution or some other injury. In a very thin cross-section of a white pine needle, you will observe many different cell types, each with a particular function.

In this activity, you will concentrate on a few major cell types and the tissues they form and some of the damage symptoms they may exhibit in an unhealthy state. As you study the terms below, use the diagrams provided by your teacher to become familiar with the cells and their position in a typical cross-section. Look at diagrams of both healthy and unhealthy cells for characteristic conditions.

Some Important Anatomical Terms**:**

A needle contains the three main tissues found in any leaf: the protective epidermis, the photosynthetic mesophyll and the conductive tissue.

Epidermis—The epidermis forms a continuous layer of cells, containing stomatal openings, around the outside surface of the needle. The epidermis is covered by a thin, waxy coating called the cuticle. The cuticle helps protect the epidermal layer as a barrier to water loss from the needle.

Stomata—The stomata are small openings in the epidermis of the needle. They are the site of gas exchange between the green inner portion of the needle (the mesophyll cells) and the air outside of the needle. The stomata are formed by two cells called guard cells that can change shape as they gain or lose water pressure. The changing shape of the guard cells opens and closes the stomata for gas exchange with the atmosphere.

Intercellular air space—You may notice these dark areas in cross-section connected to and in the vicinity of the stomata (substomatal cavity). The air and water vapor that moves in and out of the stomata moves within these spaces. The spaces eventually reach the mesophyll cells where gases can be exchanged for cellular processes such as photosynthesis.

Mesophyll Cells—These make up the green tissue seen in a cross sectional view of a white pine needle. They occupy the space between the vascular cylinder in the center of the needle and the epidermis on the outside. Mesophyll cells contain chloroplasts and are the site of photosynthesis in the needle. Mesophyll cells found in the vicinity of the stomata are often the first cells to be damaged by gaseous pollutants. These gases may react with both the cell and chloroplast membranes causing injury and loss of function. Severely damaged cells may lose chloroplasts and appear yellow or brown instead of bright green. Notice that the mesophyll cells have cell walls that are folded into the center of the cell. This increases the reactive surface area of these cells.

Chloroplasts—These organelles, found within the cytoplasm of the mesophyll cells, contain the green pigment chlorophyll. Healthy chloroplasts are vital to the process of photosynthesis. In a single cell, you should be able to see several very small oval, green structures. These are the chloroplasts.

Endodermis—This ring of cells forms a continuous layer surrounding the vascular cylinder. The cells of the endodermis serve as a differentially permeable barrier filtering the materials that move into and out of the vascular bundle.

Vascular Bundle—This tissue occupies the center of the cross-section of a needle. The xylem, phloem and fibrous support cells are contained within the vascular tissue of the bundle. Vascular tissue is continuous throughout a plant. Xylem transports soil nutrients and water from the roots to all parts of the plant including the needles. Phloem carries sugar, the product of photosynthesis, from its origin in the mesophyll out to other parts of the plant.

Plasmolysis—This condition is a symptom of possible ozone damage to mesophyll cells. Plasmolysis takes place as a result of water loss through damage to the cell membrane. This loss of water causes the membrane to pull away from the cell wall. When plasmolysis occurs, the photosynthetic capacity of the needle may be greatly diminished. In extreme cases, the cells appear empty and/or light green and have no photosynthetic capacity at all.

Resin duct—These canals carry a sticky, pungent fluid secreted by cells lining the duct. Resin inhibits the growth of certain disease causing organisms and so helps protect an injured tree from disease. In cross-section, you may notice two or three of these large circular ducts.

Nucleus—This small, membrane-bound organelle is found in every cell. It contains the cell's DNA and controls the cell's activities. You may or may not be able see the nucleus, even under high magnification, because it is very small.

Cell wall—This organelle is a rigid covering enclosing the cells of plants. It is located outside the plasma membrane and is made up of materials secreted by the cell through its membrane. The cell wall is quite porous, allowing water and dissolved substances to pass freely through it.

Discussion Questions

1. Where does photosynthesis take place in the needle?
2. How are needles protected from drying out?
3. Where do gases such as carbon dioxide and ozone enter a needle?
4. Where does the water that is used in photosynthesis come from?

NEEDLE CROSS-SECTIONS

OPTIONAL PROTOCOL

Can you observe cellular damage in a white pine needle cross-section?

In this study you will make cross-sections of a white pine needle and study the cellular condition of the needle using a compound microscope. You will choose some needles that appear to you to be healthy and some needles that appear to be damaged by chlorotic mottle and tip necrosis. The needles should range from very green, healthy needles to those with yellow to brown sections on them. You should study the various cell types and tissues in the needle cross-section, but you will be focusing mainly on the cells involved with photosynthesis, the mesophyll cells. You will study a range of cell conditions from very healthy, fluid-filled cells to yellow or brown, shrunken, or even dead (necrotic) cells. Healthy mesophyll cells are usually green due to abundant chlorophyll. Damaged cells may show a condition called plasmolysis and in extreme cases a total loss of chloroplasts, which leads to brown discoloration. A yellow coloration is due to a loss of some, but not all, of a cell's chloroplasts.

Materials

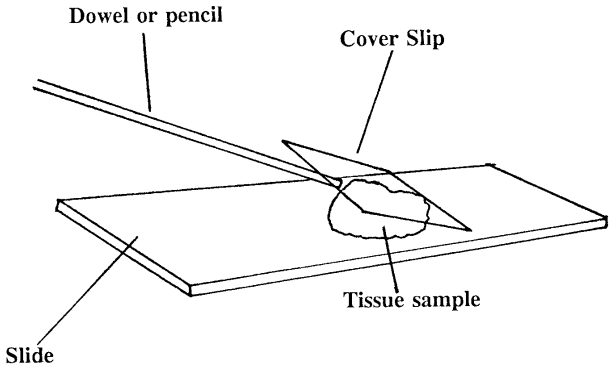
- white pine needles
- cover slips
- dissecting needle or common pins
- petri dishes with water
- dropper pipettes
- glycerin:water (1:1)
- microscope slides
- toothpicks
- microscope
- razor blades
- carrots

Procedure

To begin this activity, you will need to construct a "carrot sandwich" to help you make very thin sections from living or once living needles for microscopic observation. This procedure is demonstrated in the video on anatomy. Your instructor will also demonstrate this procedure for you.

1. Obtain a section of a carrot that measures about 5-8 cm long. Slice the carrot in half lengthwise. On the flat side of one of the carrot halves, cut a narrow notch along the axis of the carrot from end to end by dragging a dissecting needle or pin through the carrot tissue. The notch should be just large enough to fit the size of a pine needle. Alternatively, carefully cut the notch on the flat side of the carrot using a razor blade. Do not make a notch in the other half of the carrot.
2. Place a healthy white pine needle in the notched half of the carrot. Position the needle in the carrot so that you will be able to cut thin sections only through healthy areas of the needle. Place the other half of the carrot on top to form a carrot-needle sandwich. (Masking tape can be wrapped tightly around the carrot to hold the two halves in place.)

3. Even out the top of the carrot-needle sandwich by making a thin, straight crosswise cut. This should create a flat top to the sandwich. Discard this first section as it will undoubtedly be too thick to observe under the microscope.
4. Now, make very thin cross-sections by using a slicing motion with the razor blade, as demonstrated in the video, until you have 10-20 sections from which to choose. Float the cross sections in a petri dish containing a small amount of water. In this way the cross-sections will not dry out and the best of the tiny, floating, cross-section triangles can be seen.
5. Use a pipette or dropper to place a small amount of 1:1 glycerin/water solution onto a clean slide.
6. With the aid of a toothpick or pencil eraser, slide a cross-section to the edge of the water in your petri dish and transfer it onto the drop of solution on the slide. Place several (at least 8-10) cross-sections on the slide. Position the sections carefully and avoid squashing them with the toothpick or pencil!
7. Carefully place the coverslip over the cross-sections that are floating in the glycerin/water on the slide.



8. Place the slide on the microscope stage and, under low magnification, focus on one of the cross-sections. Once the cross-section is in focus, study it to see if it is thin enough to use for higher magnification. Look at several sections and decide which one is the best. Then, using this best section, switch to higher magnification.
9. Try to identify as many of the cell types and organelles as you can on this healthy needle section. Observe all of the sections on the slide and, on the Needle Cross-Section Data Sheet, draw, label and color one of the better sections that you find. Refer to an already labeled diagram to help you identify all the structures you see.
10. Now choose a dead or dying portion of a needle that shows heavy necrotic damage. It should be brown in color. Repeat procedures 1-8 with this new needle by purposefully making cross-sections through a highly damaged length of the needle. Again draw, label and color a cross-section. In severely damaged tissue, you may find that you cannot get sections which show the characteristic triangle shape of a healthy cross-section. This is because the cells are dead and may have lost their shape and integrity.
10. Repeat this procedure again, but now choose a needle that shows less damage than the one you just sectioned. It should have yellow to very lightly brown, chlorotic areas through which you will slice your sections. Again draw, color and label a thin section.

Analysis and Interpretation

1. Did you observe plasmolysis in the healthy section of needle? If so, what did it look like?

2. Which type of damage, yellow chlorotic mottle or brown tip necrosis, has the most plasmolysis?

3. When compared with the healthy sections, is the yellowing you observe in some of your cross-sections caused by lots of yellow chloroplasts instead of green ones or by fewer green chloroplasts?

4. What other sources of damage might there be to the cells inside a white pine needle other than air pollution? Did you observe any signs of fungal infections or other plant pathogens?

5. Why is it important for scientists to observe needle cross-sections as well as whole needles when they assess damage?

6. How does air pollution get into and affect needle mesophyll cells?

7. How does cell damage affect the ability of a needle to function properly? How might this affect overall health of a tree?

8. How might a scientist interpret her results if she observed more damage symptoms on one side (north or south) than the other of a white pine tree?

NEEDLE CROSS-SECTION DATA SHEET

Students/Class:_____ Date: _____

School:_____ Teacher:_____ Town:_____

Tree #'s:_____ Site Coordinates (long. and lat.):_____

Draw and label your needle cross sections below. Include one section from each of the 3 slides that you made (healthy, damaged necrotic and damaged chlorotic)

It is not necessary to send your drawings to UNH.

NEEDLE ANATOMY AND CROSS SECTIONS

C

TEACHER RESOURCE

Introduction

Students will observe the surface of needles for evidence of external damage. They will make ultra-thin cross-sections from a healthy (green) area, a necrotic (brown) area, and areas that exhibit chlorosis or yellowing on a needle. Students will then compare these cross-sections by observing them under a compound light microscope.

Guiding Question

How can you tell whether a needle cross-section exhibits cellular damage?

Materials

- white pine needles
- cover slips
- dissecting needle or common pins
- petri dishes with water
- dropper pipettes
- glycerin:water (1:1)
- microscope slides
- toothpicks
- microscope
- razor blades
- carrots

Background

In order to understand how tropospheric ozone, and/or other stress factors, may damage the cells of a needle, it is important to become familiar with the structure and function of the major cell and tissue types found in a healthy white pine needle. As you become familiar with the appearance of cells in a healthy needle, you will be better able to spot symptoms of damage in needles that may be due to ozone pollution or some other injury. In a very thin cross-section of a white pine needle, you will observe many different cell types, each with a particular function.

In this activity, students will concentrate on a few major cell types and the tissues they form and some of the damage symptoms they may exhibit in an unhealthy state. As they study the terms, have them use the diagrams to further become familiar with the cells and their position in a typical cross-section. Look at diagrams of both healthy and unhealthy cells for characteristic conditions.

Some Important Anatomical Terms**:**

A needle contains the three main tissues found in any leaf: the protective epidermis, the photosynthetic mesophyll and the conductive tissue.

Epidermis—The epidermis forms a continuous layer of cells, containing stomatal openings, around the outside surface of the needle. The epidermis is covered by a thin, waxy coating called the cuticle. The cuticle helps protect the epidermal layer as a barrier to water loss from the needle.

Stomata—The stomata are small openings in the epidermis of the needle. They are the site of gas exchange between the green inner portion of the needle (the mesophyll cells) and the air outside of the needle. The stomata are formed by two cells called guard cells that can change shape as they gain or lose water pressure. The changing shape of the guard cells opens and closes the stomata for gas exchange with the atmosphere.

Intercellular air space—You may notice these dark areas in cross-section connected to and in the vicinity of the stomata (substomatal cavity). The air and water vapor that moves in and out of the stomata moves within these spaces. The spaces eventually reach the mesophyll cells where gases can be exchanged for cellular processes such as photosynthesis.

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Chloroplasts—These organelles, found within the cytoplasm of the mesophyll cells, contain the green pigment chlorophyll. Healthy chloroplasts are vital to the process of photosynthesis. In a single cell, you should be able to see several very small oval, green structures. These are the chloroplasts.

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Plasmolysis—This condition is a symptom of possible ozone damage to mesophyll cells. Plasmolysis takes place as a result of water loss through damage to the cell membrane. This loss of water causes the membrane to pull away from the cell wall. When plasmolysis occurs, the photosynthetic capacity of the needle may be greatly diminished. In extreme cases, the cells appear empty and/or light green and have no photosynthetic capacity at all.

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Cell wall—This organelle is a rigid covering enclosing the cells of plants. It is located outside the plasma membrane and is made up of materials secreted by the cell through its membrane. The cell wall is quite porous, allowing water and dissolved substances to pass freely through it.

Management Suggestions

This activity is best used in conjunction with the anatomy video. Both the instructor and students should observe the video prior to attempting the activity.

Before beginning this activity, review general plant and animal cell structure and function with your class. Discuss with you students the various levels of organization in livings: organs, tissues and cells. You may want to discuss in more detail how fluids move up and down the various tissues in a plant. Remind students that it is at the cellular level where air pollutants do the damage that will ultimately affect photosynthetic function and health of a tree.

Take a few minutes to study the labeled diagram of the needle cross-section and the photographs of healthy cells and damaged cells at the end of this teacher resource section. In this study, we are looking at the plant's response to stress, for example water loss or exposure to tropospheric ozone. Damage, in the form of wilting or chlorotic mottle is the response we may observe. The cells that students will be observing for air pollution damage are the chloroplast bearing cells that are normally found in the green mesophyll. The mesophyll may make up the bulk of the cross section. One damage symptom that we are concerned with in this exercise is chlorosis, or the loss of chloroplasts and/or the degradation of chlorophyll within the mesophyll. Mesophyll cells that have been affected by pollutants will have a lighter green or yellow color, rather than a bright green color.

Other noteworthy features to look for on the needle cross-section diagram and/or the students' cross-sections are:

- The dark areas below the stomatal openings along one or two sides of the needle cross-section. These air-filled areas are called substomatal cavities.
- Mesophyll cells under water stress may become plasmolyzed. Plasmolysis is a condition in which the cell membrane pulls away from the cell wall due to water loss. This results in an empty space just inside the cell wall.
- If the mesophyll tissue is necrotic or dead, it will be brown in color.
- If the necrotic tissue has a network of threads running throughout, it has been parasitized by a fungus. These threads are known as stolons or hyphae and are the root-like structures used by fungus to absorb nutrients from the host organism on which it is living.

Teacher Support Materials**:**

- 35 mm slides of needle cross-sections through healthy, necrotic and chlorotic damage.
- Color images of damage symptoms on white pine needles.
- How-to video for anatomy

Use the anatomy video as an introduction for your students and have it available for the duration of the activity. It may be helpful to have students complete some additional background reading on plant anatomy and physiology as well as to label the various cell types on the photographs of the needle cross-sections before they make their observations. This will help them to understand what they are looking at when they make their microscopic observations. Practice making cross-sections of needles a few times before you do this activity with your sample needles. Students will become more confident with the technique as they try it more and more. Some students, however, continue to find sectioning both difficult and frustrating. Have students who feel comfortable pair up with those who are having trouble.

Make sure the students understand that a good needle cross-section is thin (like a slice of thin ham) as opposed to thick (like a chunk of a loaf of bread). The thin cross-sections are triangular in shape.

Try to use new razor blades each year. Make sure that you count the number of razor blades passed out and collected prior to students leaving the classroom. Have all of the blades accounted for and disposed of properly.

Time required for the activity will vary depending upon the depth to which you explore plant anatomy and physiology. Making the cross-sections and completing the observations may require up to 90 minutes.

It may be helpful to make transparencies of the photographs for use with your class. Through lecture/ discussion, have students identify the cell types and describe their function. Using the transparencies of healthy and damaged needle cross-sections, have students describe the damaged areas as they look for cells that may exhibit plasmolysis.

Reporting the Results:

You are not required to send copies of this activity to UNH. You may keep student drawings for your records. But, if you have a camera system attached to your microscope and you get good photos of cross-sections, you can send these to us and we'll place them on the web site.

For Further Study**:**

Ask students to develop a hypothesis as to why particular cells become damaged. For example, one would expect that mesophyll cells adjacent to stomata are the first to be damaged because this is the area of gas exchange between the internal needle and the ambient air.

Some of the more outstanding student cross-sections can be taken to a local college or lab where they can produce a photomicrograph of the slide. This photo image can then be used in the future to teach others about needle structure and proper sectioning technique.

Cutting through the needles with the razor blade may open up one or more resin ducts found in the needle. Students may detect the odor of the fragrant resin that provides both insect and frost protection for the tree.

Your students may wish to compare pine needle anatomy to deciduous leaf anatomy. Use a hand lens or stereomicroscope to examine the upper and lower surfaces of deciduous leaves. Consult a biology or botany text for labeled illustrations of the deciduous leaf anatomy.

Answer Key

1., 2., 3. Answers will vary depending on student results. Some students will be discouraged because they are not able to easily or successfully obtain cross-sections. Encourage students to share better sections for observation and discussion. You should probably discuss the kinds of observations you might expect to see at the various levels of damage with the class as a group. Students should be able to answer questions 4 through 8 even if they are not successful with their own sectioning technique.

4. What other sources of damage might there be to the cells inside a white pine needle other than air pollution? Did you observe any signs of fungal infections or other plant pathogens?

   Other sources of damage might include infection by fungi or other pathogens, frost damage, desiccation, insect or other animal injury. If the necrotic tissue has a network of threads running throughout, it may have been parasitized by a fungus. These threads are known as stolons or hyphae and are the root-like structures used by fungus to absorb nutrients from the host organism on which it is living.

5. Why is it important for scientists to observe needle cross-sections as well as whole needles when they assess damage?

   The more information that scientists can collect about their samples the better. External needle observations may not be enough to indicate the exact nature of an injury. For example, the presence of fungal hyphae under microscopic observation tells us that necrotic damage may be due in part, at least, to fungal infection rather than some other impact such as air pollution.

6. How does air pollution get into and affect needle mesophyll cells?

   Gas exchange, and therefore the entrance of air pollutants, occurs through the stomata which are actually small openings in the epidermis of the needle. They are the site of gas exchange between the green inner portion of the needle (the mesophyll cells) and the air outside of the needle. The stomata are formed by two cells called guard cells that can change shape as they gain or lose water pressure. The changing shape of the guard cells opens and closes the stomate for gas exchange with the atmosphere.

   Mesophyll cells contain chloroplasts and are the major site of photosynthesis in the needle. Mesophyll cells found in the vicinity of the stomata are often the first cells to be damaged by gaseous pollutants. These gases may react with both the cell and chloroplast membranes causing injury and loss of function. Severely damaged cells may lose chloroplasts and appear yellow or brown instead of light green.

7. How does cell damage affect the ability of a needle to function properly? How might this affect overall health of a tree?

   Damage to or loss of chloroplast function in the mesophyll cells will negatively impact the photosynthetic capacity of the needles. If many needles are impacted, then there is less food production overall and the general health of the tree may decline.

8. How might a scientist interpret her results if she observed more damage symptoms on one side (north or south) than the other of a white pine tree?

   Factors might include: microclimate differences between north and south sides of the tree, water availability, differential exposure of the two sides to harsh environmental elements, i.e. prevailing winds, cold winter blasts and/or air pollutants, or the south side of a tree receiving more sunlight than the shaded north resulting in increased photosynthetic activity, which in turn may exacerbate damage if ozone or other air pollutants are present.

SPECTROPHOTOMETRY

BACKGROUND

Guiding Question

What can information about light transmittance through a chlorophyll extract tell you about the health of a needle?

All energy that exists on the earth and in the universe is a part of the electromagnetic spectrum. Energy in this spectrum can be described by its frequency and by its wavelength. Wavelength is the distance between the peaks or tops of a moving wave. Very long radio waves can be 200 meters long while the distance between extremely short gamma wave peaks is less than the diameter of an atom.

Wave frequency is defined as the number of wave cycles (the number of peaks that pass a point) per second (also called Hertz). The frequency of a radio wave is very low while the frequency of a gamma ray is very high. The relationship between wavelength and frequency is inversely proportional, as the wavelength increases the frequency decreases. Another way to think of this relationship is that as the wavelength decreases the number of energy waves that are hitting an object per second increases.

As you read this paragraph, the long radio waves that are in the air around you are actually bouncing right off you, doing you no harm. This is because of their very low frequency. Step inside a nuclear reactor though, and the short wavelength gamma rays produced by the nuclear reaction hit you with such frequency that they can burn holes through your cells. If you stay in that reactor long enough, these gamma rays will eventually kill you.

Living organisms use the sun's energy in many life processes. One portion of the electromagnetic spectrum that is important to living organisms is the visible light portion. Plants are capable of using some of the energy in the visible portion to manufacture food through a process called photosynthesis. Chlorophyll, a plant pigment, captures this visible light energy and releases the energy in a series of chemical reactions to complete photosynthesis.

The chemical equation depicting photosynthesis is as follows:

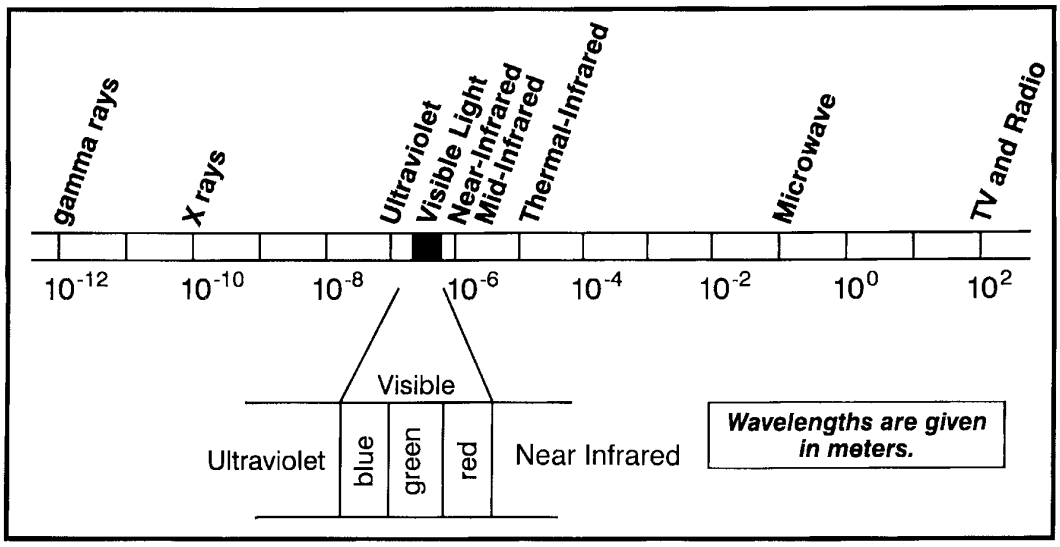
$$6CO_2 + 12H_2O \xrightarrow[\text{Visible light}]{\text{Chlorophyll}} C_6H_{12}O_6 + 6H_2O + 6O_2$$

In order for a plant to remain healthy it must be able to photosynthesize properly. Ozone gas, as well as other environmental factors, has the capability of damaging cells and cell parts associated with photosynthesis. The loss of chlorophyll in cells reduces a plant's ability to photosynthesize properly and over time may result in premature death.

An instrument known as a Spectrophotometer-20 (Spec-20) is able to measure the relative absorption or transmission of light energy as it passes through a liquid in a small test tube. We will measure the amount of visible light that is transmitted through a chlorophyll solution that has been extracted from our needle samples. Some of the visible light wavelengths will be absorbed while other light wavelengths will be transmitted through the solution. The information is recorded as % transmission. Upon inspection, you should notice that the graph of the transmission spectrum of the chlorophyll could be inverted to give the peaks and troughs of an absorption spectrum. This makes sense since a liquid with a high transmission at a given wavelength has little absorption.

The Spec-20 analysis of the chlorophyll in a white pine needle can be used as an indicator of the relative amount of the chlorophyll occurring in a needle. The results of the chlorophyll analysis can also be used in conjunction with other tests for ozone damage such as the spectral results of the VIRIS scans (reflectance measurements), cellular and visual needle observations, wet-weight/dry-weight analysis and so forth. This combination of analyses will help provide a more comprehensive view of the health of your pine tree.

Electromagnetic Spectrum



Wavelengths of visible light:

Blue visible light: 4.5×10^{-7} meters
Green visible light: 5.5×10^{-7} meters
Red visible light: 6.5×10^{-7} meters

Discussion Questions

1. What is the electromagnetic spectrum?

2. Distinguish between wavelength and wave frequency.

3. List some types of electromagnetic energy.

4. How do plants use the sun's energy?

5. How might ozone interfere with the photosynthetic process in a plant?

6. How will you use the Spectrophotometer20?

SPECTROPHOTOMETRY

C

OPTIONAL PROTOCOL

Chlorophyll is a substance found in the cells of plants that helps the plant capture the sun's radiant energy and transform it into the chemical energy that is necessary for the plant to survive. Other organisms, in turn, may consume that plant and the matter and energy can be transferred.

Scientists know which wavelengths of energy are captured by the chlorophyll in the cells of green plants through the process of photosynthesis. If chlorophyll cannot function in the cells of the plant, energy capture will not take place. To help us understand how chlorophyll functions, we can take the chlorophyll out of the needles and artificially measure how it uses light energy. The instrument that allows us to make this measurement is called a spectrophotometer.

The visible portion includes the waves of light that we can actually see. They range from the shortest violet waves to the longest red waves. The "colors of the rainbow" are found in between these extremes. From the visible portion of the electromagnetic spectrum, chlorophyll absorbs energy in the blue and red wavelengths. What color of visible light is reflected from the needles of white pine trees?

In this activity, you will learn how to extract and make a solution of the chlorophyll from the cells of pine needles. You will use a spectrophotometer to measure how the chlorophyll solution transmits light at various wavelengths in the visible portion of the electromagnetic spectrum.

Materials

- white pine needles from north and south quadrants
- cuvettes
- Kimwipe cleaning tissues
- grease pencil
- balance
- test tubes and test tube racks
- freezer
- parafilm (cut into several 4x4 cm sections)
- razor blade
- 80% acetone:20% distilled water solution

CAUTION: Acetone is a flammable, poisonous solvent. Wear eye protection. Use fume hood, if possible. Razor blades are sharp!

Procedure

Day 1: Extracting chlorophyll from needle sample

1. Obtain a small sample of a branch from either the north or south quadrant storage bag. Close the bag quickly and note which quadrant you are sampling from on your data sheet! Return the bag to the cooler or refrigerator immediately.
2. From your small sample, remove 15-20 needle fascicles.
3. Place the needles onto a clean piece of paper and, using the razor blade, carefully slice the needles into sections 3-5 mm long. Do not include the brown sheath material.
4. Use a balance to accurately mass 1.0 grams of sliced needle sections.
5. Transfer the 1.0 g of sliced needles to a test tube labeled with the tree # and quadrant you are preparing.
6. Slowly add 6.0 ml of 80% acetone solution to the test tube. Seal the tube with two layers of parafilm.
7. Place the test tube with needle extract sample in a freezer for 24 hours.

Day 2: Spectral analysis of chlorophyll extract

A. Complete the sample preparation:

1. Remove the extract test tube from the freezer and allow it to warm up to room temperature. Your teacher may already have done this for you.
2. While the test tube is warming up, turn on the spectrophotometer and let it warm up as well. Your teacher may already have done this for you.
3. Pour 3.0 ml of the room temperature extract solution into a graduated cylinder.
4. Add 9.0 ml of 80% acetone solution to make a 3:1 dilution.
5. Cover the cylinder with parafilm and, holding the parafilm in place, invert several times to mix.
6. Transfer 6.0 ml of the mixture into a cuvette labeled with the appropriate identification. (Use a grease pencil to label the top of the cuvette so it will not interfere with the spectral analysis or label the test tube rack position as to the tree and quadrant that corresponds to the sample in the cuvette being held in that position.)

B. Spectral analysis of extract solution:

This section includes general instructions for operation of the Spec-20D model spectrophotometer. Your teacher may ask you to consult the operating instructions that accompany the model spectrophotometer you are using for further clarification.

1. Use the diagram in Fig. 2 of **Key Operating Features of the Spec 20** that came with the instrument to help you complete the following instructions.
   a. Select wavelength at 400 nm using the wavelength control.
   b. Set the mode to TRANSMITTANCE (press the MODE select control until the transmittance LED on the right of the display is lit).
   c. With the sample compartment empty and cover closed, adjust Zero Control so that the meter reads 0%T.
   d. Insert a reference blank (clear 80% acetone solution) cuvette into the sample compartment and set transmittance to 100%T. Remember to match the white line on the cuvette with the line on the sample chamber. Close the cover.
   e. Remove the reference blank and insert the cuvette containing the chlorophyll extract into the sample compartment. Close the cover.
   f. Read the measurement from the display in % transmittance and record it on your spectral results data sheet.
   g. Change the wavelength adjustment to 425 nm and repeat steps c through f.
   h. Repeat steps c through f, changing the wavelengths by 25 nm increments until you have data up to and including 700 nm.
2. The data from your spectral results data sheet can be used to create a graph of the % transmittance results on the grid provided with this activity. Obtain data from the opposite quadrant for your tree and, using colored pencils, graph both the north and south lines onto one graph. Label your lines or make a key identifying north and south.

SPECTROPHOTOMETRY DATA SHEET

Students/Class:_____ Date: _____

School:_____ Teacher:_____ Town:_____

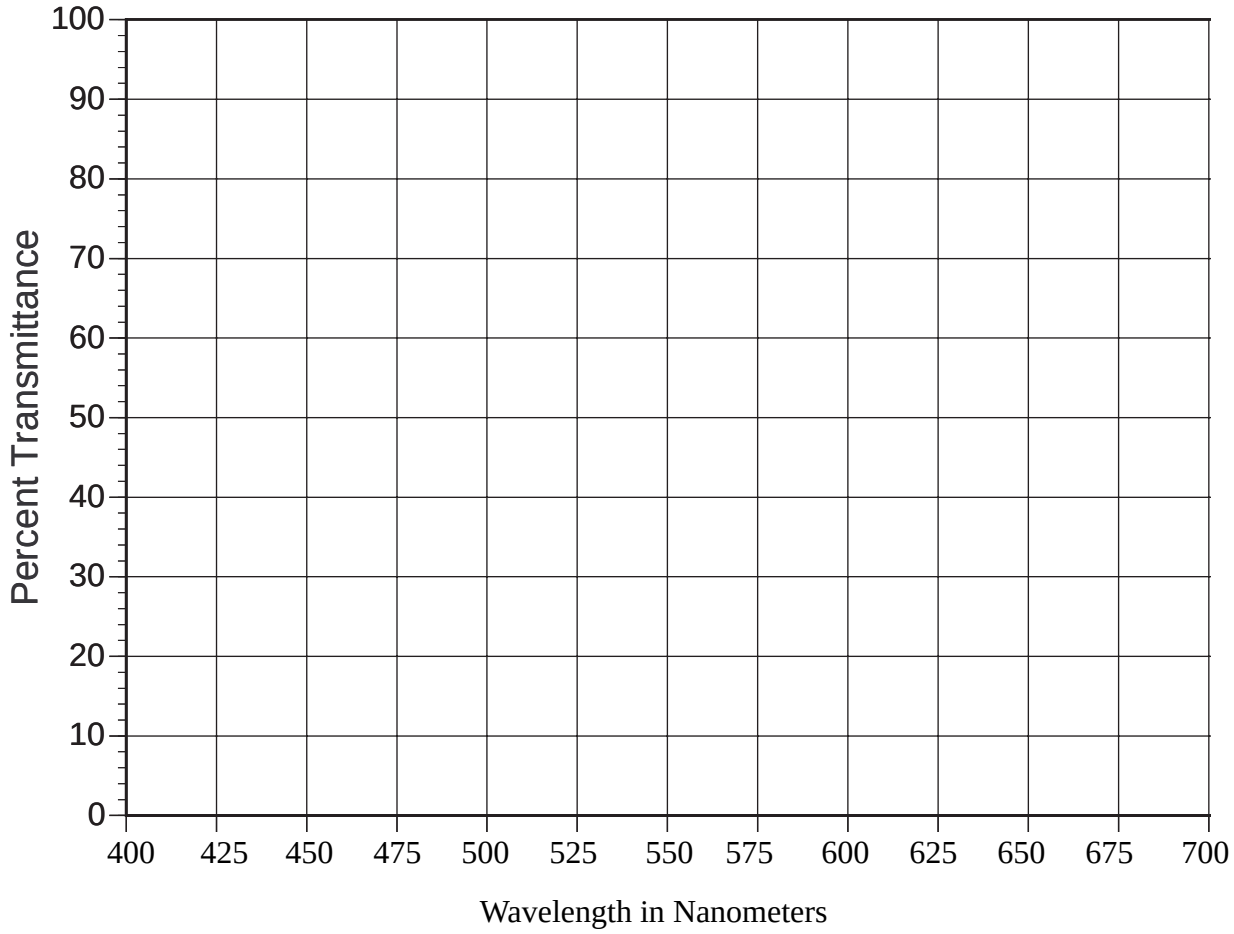
Tree #'s:_____ Site Coordinates (long. and lat.):_____

Needle mass used: _____

Dilution (Acetone/Water: Extract): _____

Wavelength	North % Transmittance %transmission N	South % Transmittance % transmission S
425		
450		
475		
500		
525		
550		
575		
600		
625		
650		
675		
700		

Note: You do not need to send this data sheet to UNH. If you wish, send a class-averaged date sheet and graph for each sample.


Percent Transmittance
100
90
80
70
60
50
40
30
20
10
0
400
425
450
475
500
525
550
575
600
625
650
675
700
Wavelength in Nanometers

Analysis

1. Compare the % transmission lines for the north and south data for your tree. What are the similarities and differences you observe?

2. How does the % transmittance of light change as you move from 400 nm to 700 nm?

3. Which wavelengths are associated with the peaks and troughs of your graph?

4. What colors do these represent on the visible spectrum?

5. Explain what is happening to the light at the peaks and troughs.

6. One way that you can assess the relative amount of the chlorophyll in your samples is to compare % T lines between different samples. A line that is shifted upwards from the norm indicates greater transmittance at those wavelengths. How might you interpret this information with respect to chlorophyll?

7. If the graphs for the north and south quadrants for your data are not exactly the same, speculate as to which quadrant may have less chlorophyll. Explain your answer.

8. What other factors could have affected the results you obtained?

Conclusion

Relate the % T results you have obtained in this activity to the information you have already learned about the effects of tropospheric ozone on the photosynthetic cells of a plant. Use the information you have gathered concerning needle damage (whole needle and cross-sections), % water results, and any other pertinent data or observations to make a statement about the health of the sample needles from your tree and the possible affects of ozone on your tree. Be sure to note any differences between the quadrants and speculate as to why those differences might exist. Ask your teacher for any information he or she might have about actual ozone measurements in your area for last summer.

SPECTROPHOTOMETRY

TEACHER RESOURCE

Introduction

Students are introduced to the electromagnetic spectrum and the light energy that is used by plants during photosynthesis. Analysis of which wavelengths of light energy are absorbed and transmitted by chlorophyll in white pine needles is accomplished through the use of a Spectrophotometer 20. Students create and interpret spectral graphs of the % transmittance data obtained from the Spec 20 and attempt to relate their results to the health of their tree and the possible effects of ozone pollution.

Guiding Question

What can information about light transmittance through a chlorophyll extract tell you about the health of a needle?

Materials

- white pine needles from north and south quadrants
- cuvettes
- Kimwipe cleaning tissues
- grease pencil
- balance
- test tubes and test tube racks
- freezer
- parafilm (cut into several 4x4 cm sections)
- razor blade
- 80% acetone:20% distilled water solution

CAUTION: Acetone is a flammable, poisonous solvent. Wear eye protection. Use fume hood, if possible. Razor blades are sharp!

Background

All energy that exists on the earth and in the universe is a part of the electromagnetic spectrum. Energy in this spectrum can be described by its frequency and by its wavelength. Wavelength is the distance between the peaks or tops of a moving wave. Very long radio waves can be 200 meters long while the distance between extremely short gamma wave peaks is less than the diameter of an atom.

Wave frequency is defined as the number of wave cycle (the number of peaks that pass a point) per second (also called Hertz). The frequency of a radio wave is very low while the frequency of a gamma ray is very high. The relationship between wavelength and frequency is inversely proportional, as the wavelength increases the frequency decreases. Another way to think of this relationship is that as the wavelength decreases the number of energy waves that are hitting an object per second increases.

As you read this paragraph, the long radio waves that are in the air around you are actually bouncing right off you, doing you no harm. This is because of their very low frequency. Step inside a nuclear reactor though, and the short wavelength gamma rays produced by the nuclear reaction hit you with such frequency that they can burn holes through your cells. If you stay in that reactor long enough, these gamma rays will eventually kill you.

Living organisms use the sun's energy in many life processes. One portion of the electromagnetic spectrum that is important to living organisms is the visible light portion. Plants are capable of using some of the energy in the visible portion to manufacture food through a process called photosynthesis. Chlorophyll, a green plant pigment, captures this visible light energy and releases the energy in a series of chemical reactions to complete photosynthesis.

The chemical equation depicting photosynthesis is as follows:

$$6CO_2 + 12H_2O \xrightarrow[\text{Visible light}]{\text{Chlorophyll}} C_6H_{12}O_6 + 6H_2O + 6O_2$$

In order for a plant to remain healthy it must be able to photosynthesize properly. Ozone gas, as well as other environmental factors, has the capability of damaging cells and cell parts associated with photosynthesis. The loss of chlorophyll in cells reduces the plant's ability to photosynthesize properly and over time may result in the premature death.

An instrument known as a Spectrophotometer-20 (Spec-20) is able to measure the relative absorption or transmission of light energy as it passes through a liquid in a small test tube. We will measure the amount of visible light that is transmitted through a chlorophyll solution that has been extracted from our needle samples. Some of the visible light wavelengths will be absorbed while other light wavelengths will be transmitted through the solution. The information is recorded as % transmission. Upon inspection, you should notice that the graph of the transmission spectrum of the chlorophyll can be inverted to give the peaks and troughs of an absorption spectrum. This makes sense since a liquid with a high transmission at a given wavelength has little absorption.

The Spec-20 analysis of the chlorophyll in a white pine needle can be used as an indicator of the relative amount of the chlorophyll occurring in a needle. The results of the chlorophyll analysis can also be used in conjunction with other tests for ozone damage such as the spectral results of the VIRIS scans (reflectance measurements), cellular and visual needle observations, wet-weight/dry-weight analysis and so forth. This combination of analyses will help provide a more comprehensive view of the health of your pine tree.

Management Suggestions

It is important to measure the transmission spectrum of chlorophyll while the chlorophyll in the needle sample is in a normal "light absorbing" state. The chlorophyll will remain in this state for several weeks if the needles are wrapped in wet paper towels, sealed in a plastic bag and stored in a refrigerator or freezer.

Once the needle/acetone extraction process has begun, however, the Spec-20 testing must be done in the 24-hour time frame indicated in the lab procedure. It is important, therefore, to plan this as a definite two-day activity. Since not all students will be able to participate in this activity at the same time, you may have a variety of needle analysis activities going on in class.

The key to this multi-activity approach is training and preparation of the students beforehand, so when you are ready to go with the real samples the class will run as smoothly as possible. By this time, students should have practiced external needle measurements, cross-sections, etc. and will be well prepared to go right to work. Preparation for the Spec-20 tests can be accomplished through some practice exercises as well. See the section "Additional Suggestions" for some ideas.

If possible, to facilitate an understanding of the concepts in this section, students should be introduced to the following ideas before beginning the lab:

- An introduction to the electromagnetic spectrum (visuals an absolute necessity)
- The importance of chlorophyll in the process of photosynthesis

Before beginning this activity, teachers should:

- Prepare stock solutions of 80% acetone:20% distilled water.
- Become familiar with the use of the Spec-20 (possibly working with a chemistry teacher).
- Have all materials clean and ready to go before the activity begins.
- Count the razor blades before class and at the end of class before the students leave to ensure safety for all.

Additional Suggestions

1. Use a diffraction grating demonstration to help illustrate the properties of light.
2. **Activity: Introduction to the Spectrophotometer 20** in which students generate data and graphs for red, green, and blue food-colored water. This will help train them in the use of the instrument as well as expose students to spectral curves.
3. Try a chromatography experiment with the chlorophyll sample extract.
4. Hold the extracted chlorophyll in the sunlight to show fluorescence.

Time to Complete Activities

One class or a double lab to set up Day 1 extraction. At least 1 class to complete Day 2, but very likely the process will take more than one class to complete. The instructor may have to complete the analysis or arrange for some students to work into an extended time period. If more than one Spec-20 is available then the analysis will go more quickly. One period to share and analyze data in class. Students may complete questions for homework.

Evaluation: Applying what you have learned

A greenskeeper, hired to grass over a golf course, wanted to determine which type of grass seed to plant in the sandy soil of the course. She knew that chlorophyll was necessary for the grass to be healthy and grow well. As a consultant for a landscaping firm you have been asked to help her decide which grass to grow. Design an experiment in which you obtain spectral data to help her determine the best choice.

(Possible answer: Grow several seed types in sandy soil. Perform a spec-20 analysis on the grass and compare the resultant spectral curves with a typical plant transmission spectrum.)

Note:

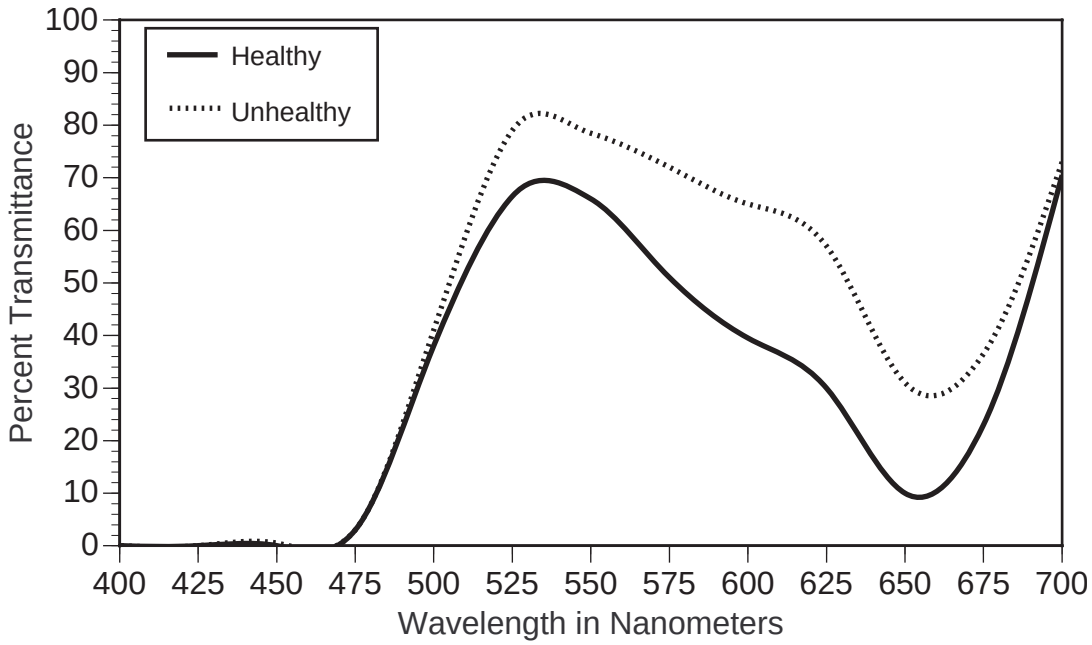
a. You may wish to point out to your students the importance of using an Exact Measure of Needle Mass and Solvent Volumes in order to make valid between-sample comparisons.
b. These chlorophyll data are not absolute concentrations because full Segment Extraction is only possible with the use of dangerous solvents and precise methods. This Protocol, though, provides a relative comparison of chlorophyll between samples which are processed the same time in exactly the same manner. This protocol illustrates, very well, chlorophyll absorption of light energy.
c. These data are not directly used by UNH, but if your class wishes to send us one class-averaged transmission graph for each sample, this would be useful and we can add these data/graphs to the web site database. Please document the exact time, solvent values, and other methods used.

Answer Key

Analysis and Conclusions

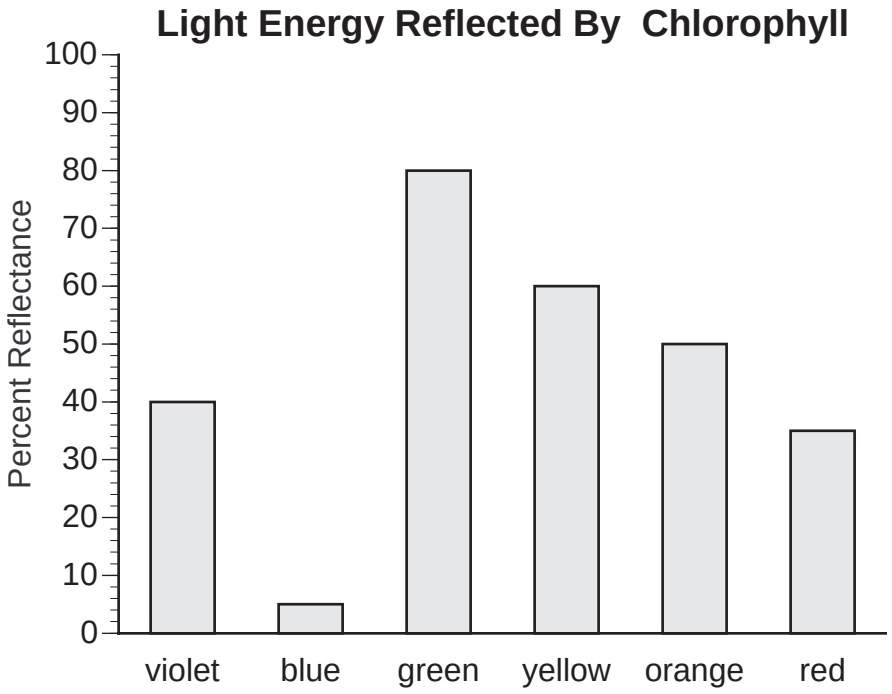
Student answers will vary depending on their results and the graphs they create.

Analysis of the transmission graphs generated by students will most likely show some differences either between quadrants and/or between sample trees. Some differences to look for might include an upward shift of the red and to some degree the blue portion of the line. This shift, then, creates a widening or rounding out of the portion of the graph near the green wavelengths. These changes signal that there may be relatively less chlorophyll in that sample, possibly because there was relatively less chlorophyll in the needle from which it came. More light is being transmitted, or alternatively, less light is being absorbed at those wavelengths. The graph below compares chlorophyll extracts from healthy and damaged needles.



The highest % transmission through the chlorophyll extract solution is between 500-600 nm. This corresponds to green color. It follows then that we see plants as green because the chlorophyll of intact leaves reflects the light in the green portion of the electromagnetic spectrum. The light wavelengths that are least transmitted and used most in the process of photosynthesis are between 400-475 nm, the violet to blue light wavelengths, and between 625-675 nm, the red light wavelengths. Because the energy in these wavelengths is being absorbed and not reflected by the plants, we do not see leaves as blue or red. (Students may ask about plants that have red or other colors in their leaves. A discussion of fall leaf color change and the presence of other leaf pigments might be appropriate at this time.)

The graph below shows the approximate percentage of light energy reflected by chlorophyll. Compare it to the transmittance curves on the preceding page.



Answers to commonly asked questions:

1. Why is it important to return your sample bag to the refrigerator as soon as possible after you obtain your sample needles?

   The reason that the unused sample is immediately placed back into the refrigerator is to prevent change in the needle sample. Not all of the procedures for the white pine study may have been completed. Refrigeration will maintain them as fresh samples for later study.

2. Why is it important that the needle extract solution stand in the freezer for as close to 24 hours as is possible for every school? What might happen if some extracts were made from less than or more than 1.0 g of needle samples? Could we confidently compare the results?

   The reason that the 1.0 grams of needle with 6 ml of 80% acetone:20% distilled water solution is allowed to stand for exactly 24 hours is to help insure an accurate and uniform Spec-20 procedure for all of the schools that are collecting these data. Controlling for needle mass and time in the freezer will allow for relative comparisons between different schools.

3. Explain the purpose of the reference cuvette.

   The blank cuvette serves as a control for the solvent used in the chlorophyll solution. The Spec-20 must be reset each time the wavelength is changed with the "blank" reference sample. Each test then becomes a comparison to the blank and not to the test samples themselves since the transmission characteristics from the previous test are removed.

INTRODUCTION TO THE SPECTROPHOTOMETER 20

ACTIVITY

What is a Spectrophotometer 20?

How do you use the Spectrophotometer 20?

A Spectrophotometer 20 (Spec-20) is an instrument that will allow you to measure the relative absorption or transmission of visible light energy through a test solution. Some of the wavelengths of light will be absorbed and some of the wavelengths will be transmitted depending on the chemical makeup of the solution. The information is recorded as % transmittance and can be used to generate a graph. Peaks and valleys on the graph will correspond to absorption and transmittance of light energy.

Part 1

This activity will familiarize you with the operation of the Spec-20. You will become familiar will the function of the instrument, the data it generates, and how to graph and interpret the data.

Materials

- spectrophotometer
- distilled water
- cuvettes
- white chalk cut to expose a slanted top

Procedure

1. The Spec-20 has been turned on and warmed up prior to class.

2. Carefully and slowly turn the large wavelength adjustment knob on the top of the Spec-20 and observe what happens on the wavelength display window. What do the numbers on the display represent?

3. For this activity, wavelength is measured in nanometers. (1 nanometer (nm) = 1 x 10 -9 meters). Adjust the wavelength to 520 nm. Open the lid of the sample compartment. Place a cuvette with chalk into the sample compartment. The slope of the chalk should face toward the wavelength control knob. (Never empty the cuvette into the container!) Look into the sample compartment and describe what you see.

4. While looking at the chalk, slowly turn the transmittance/absorbance control knob clockwise then counter-clockwise. What do you observe?

5. Adjust the transmittance control knob until you observe a bright band of light on the chalk.

6. Adjust the wavelength to the lowest possible setting. Then, watching the chalk in your cuvette, increase the wavelength until the light first appears on the chalk. This is the lowest visible wavelength. What is this wavelength?

7. Slowly turn the wavelength control knob through its entire range. Describe what you see.

8. Looking at the chalk, determine the range of wavelengths for each color of light and record the results in the Wavelength Data Table.

Wavelength Data Table

Color of Light	Wavelength (nm)	
	Low	High
Red	_____	_____
Orange	_____	_____
Yellow	_____	_____
Green	_____	_____
Blue	_____	_____
Indigo	_____	_____
Violet	_____	_____

9. Describe the relationship between color and wavelength. In your experience, where else have you seen colors appear in this order?

10. Remove the chalk cuvette and close the lid on the sample compartment. Turn the left front knob until the display is 0.0. This power switch is also responsible for setting the instrument to zero and is also called the zero control knob.

11. Fill a cuvette with distilled water to within 2 cm of the top. Since the water in this cuvette does not have anything dissolved in it, it is called a blank. Wipe the bottom of the cuvette with a Kimwipe to make sure there is no dirt on it. Always handle the cuvettes by touching them near the top by the mark. Notice that the cuvette has a line at the top; this mark should line up with the raised ridge on the front of the sample compartment. Insert the cuvette and close the lid on the compartment. Why is it important to keep cuvettes clean?

12. Adjust the transmittance control until the right number reads 100.0. This represents 100% transmittance. 100.0% transmittance equals what % absorbance? 10.0% transmittance equals what % absorbance?

13. Remove the cuvette from the sample compartment. Set the wavelength to 425 nm. Using the zero control knob, zero the instrument if it is not already on zero. Insert a blank and set transmittance to 100.0%. Adjust the wavelength to 500 nm. What has happened to the % transmittance?

14. Because the instrument is not equally sensitive to all wavelengths, it must be adjusted <u>every</u> <u>time you change wavelengths</u>. The transmittance control should be adjusted so that the instrument reads 100.0% transmittance when the blank is placed in the sample holder.

Questions

1. When there is no cuvette in the sample holder, which knob do you turn to zero the instrument?

2. In addition to the markings, how do you think the cuvettes are different from ordinary test tubes? Is there anything that has to be more carefully made on a cuvette?

3. Why was a piece of white chalk used for this lab?

4. a. What do you think the approximate wavelength is for infrared light?

   b. What do you think the approximate wavelength is for ultraviolet light?

Part 2

Colored solutions are colored because they absorb certain wavelengths of light while allowing other wavelengths of light to pass through. As observers, we see the wavelengths of light that are not absorbed. By measuring the amount of light absorbed we can find the concentration of solutions. Light that is not absorbed by the solution can be recorded as % transmittance. The data can be graphed and analyzed for its absorption characteristics.

Materials

- spectrophotometer
- red, blue, green colored water
- cuvettes

Procedure

1. Choose one of the colored water solutions. Rinse the cuvette with a small amount of colored solution and then fill a cuvette 3/4 full.
2. Fill the second cuvette with distilled water to use as a blank.
3. Set the spectrophotometer to 350 nm.
4. With the sample compartment empty and the lid closed, adjust the zero control so that the meter reads 0.0% T.
5. Place the blank into the sample compartment and use the transmittance knob to set T at 100%.
6. Remove the blank and place the colored solution into the spectrophotometer. Record the %T on the data sheet.
7. Reset the wavelength to 375 nm and repeat steps 4, 5, and 6.
8. Repeat steps 4, 5 and 6 recording transmittance every 25 nm until you reach 700 nm. (Note: the instrument that you are using may need to have the filter adjusted to reach this range. Your teacher will help you determine whether or not this will be necessary.)
9. Locate the 50 nm region in which the % transmittance was the least. This represents the greatest absorption measured. Set the instrument to this point and record the transmittance every 10 nm in this region repeating steps 4, 5, and 6.
10. Determine the wavelength of minimum transmittance and maximum absorbance.
11. Repeat this procedure for the other two colored solutions or combine your data with others.

Colored Solution Activity
Data Table % Transmittance

Solution color_____ Solution color_____ Solution color_____

Wavelength (nm)	% transmittance	Wavelength (nm)	% transmittance	Wavelength (nm)	%transmittance
350		350		350	
375		375		375	
400		400		400	
425		425		425	
450		450		450	
475		475		475	
500		500		500	
525		525		525	
550		550		550	
575		575		575	
600		600		600	
625		625		625	
650		650		650	
675		675		675	
700		700		700	

Analysis

1. The data from your spectral results can be used to create a graph of the % transmittance. Graph the information for all three solutions on the same grid. Provide a key for your graph.

2. What is the wavelength of maximum transmittance for each colored solution tested? What color of light corresponds with this wavelength?

3. What is the wavelength of maximum absorbance for each colored solution tested? What color of light corresponds with this wavelength?

4. Compare the absorption and transmittance characteristics of the three solutions.

5. Different solutions absorb light of different colors or wavelengths. What does the amount of light absorbed depend on?