Milkweed Monitoring: Keeping a Finger on Nature's Pulse

Teacher Guide

Lesson Focus:

How do biomonitoring scientists use plants and animals to track pollution and changes in ecosystems?

Objectives:

The student will be able to:

1. Recognize common milkweed and its structure.
2. Understand how ground-level ozone is formed, and how it affects common milkweed.
3. Identify ozone injury to common milkweed.
4. Collect injured and non-injured leaves, and add their results to a statewide database.

Time:

2 class periods (some classrooms have spent 2-3 weeks on this activity, treating it as a unit)

Materials:

Plant press (instructions included on how to make your own), hand lenses, kit materials (download on EEK!)

Grade Levels:

5-12

***Wisconsin Academic Standards Met:**

Mathematics: D.8.1, D.8.3, D.8.4, D.12.2, D.12.3

Science: C.8.1, C.8.3, C.8.4, C.8.5, C.8.6, C.8.8, C.12.7, D.8.3, D.12.4, F.8.8, F.12.8, G.8.3, G.12.3

***** The Wisconsin Academic Standards are designated by number only, in order to reduce the total number of pages of this activity.

Background:

Biomonitoring

In the 1800's few jobs were harder or more dangerous than an underground coal miner. Over the years thousands of men, women and children were killed in mine accidents. One common cause of the accidents was an accumulation of methane and carbon monoxide in the shafts. These gases could lead to violent explosions. Miners started to use the canary to test the air quality in the mines. Canaries acted as indicators of poor air and potentially dangerous gases.

Declining eagle populations in the 1960's led to the discovery that certain chemicals could accumulate in the environment and change natural systems. In many ways monitoring the environment can be done by closely watching plants and animals. Extensive air pollution research has shown that plants are more sensitive than animals to air pollution. There is increasing concern that air pollution is hurting the health of our forests and crops. One way to assess the impact of air pollution on plants is to document visible injury on sensitive plant species. Plants that are especially sensitive to air pollution make excellent natural environmental monitors. These plants are called bioindicators. Bioindicators are plants that reveal well-defined and consistent responses to high ozone concentrations.

Plants sensitive to ozone injury can be used as biological indicators (biomonitors) of air pollution stress in both urban and remote rural ecosystems.

For more information about biomonitoring, refer to "Vital Signs" in the *Where's the Air?* CD-ROM kit.

Ozone: "Good up high, bad nearby"

You may have heard that ozone shields us from the from the sun's harmful UV, or Ultraviolet, rays. This type of ozone is called "stratospheric" ozone. Stratospheric ozone is made up of three oxygen atoms, and has no color, no taste, and not much odor. In fact, stratospheric ozone is the same chemical as ground level ozone. The difference between stratospheric ozone and ground level ozone, a.k.a. tropospheric ozone, is where each is found.

Stratospheric ozone is found in the stratosphere, between 6 and 20 miles above the ground. The ozone in this layer of air protects plants, animals, and us by blocking the most harmful rays of the sun.

Tropospheric ozone, a.k.a. ground-level ozone, is found in the troposphere, which is the layer of air closest to the Earth's surface. The troposphere is the air from the ground to about 6 miles up into the atmosphere - it's the air we breathe. Ozone does not naturally occur at harmful levels in the troposphere. Our ground-level ozone problems are caused by human activities.

Just remember: "Good up high, Bad nearby!".

Your students may ask: we have too much ozone in the troposphere, and not enough in the stratosphere: so why can't we just send tropospheric ozone up into the stratosphere?

Unfortunately, we can't simply 'pump' our extra ozone into the stratosphere (where it could help stop harmful UV rays from reaching the Earth). So to keep it from causing problems down here in the troposphere, we have to stop it from forming in the first place.

Ground-level Ozone Formation and Transport

Cars, factories, boats, lawn mowers, paint cans, and other household products emit pollutants. Ground-level ozone forms when those pollutants bake in the hot summer sun. Because ground-level ozone is formed by combining these pollutants, it is called a secondary pollutant.

Ozone is made up of three oxygen atoms. Because of this, it is unstable and can react with things, such as rubber, plants, and even your lungs.

There are three main components needed to make ground-level ozone: NOx, VOCs, and sunlight.

NOx stands for a group of chemical compounds called Nitrogen Oxides. These chemicals are made up of nitrogen and oxygen. They are created mostly by cars and other engines powered by gasoline, like lawn mowers and motorboats.

VOC stands for Volatile Organic Compound. VOCs are hydrocarbons. This means they are made up of hydrogen and carbon. VOCs are created mostly by cars, but also by industries that burn fossil fuels, such as a coal-burning power plant. Other sources of VOCs include drying paint, charcoal lighter fluid, aerosol cans, motor boats, lawn mowers, and nail polish remover.

Sunlight is the third component. The rays in sunlight cause the nitrogen oxides and VOCs to mix together. When these chemicals combine, they form ozone and other byproducts. Air currents then scatter this ozone all over the Lake Michigan region, where we all breathe it.

For more information about ozone, refer to the *Where's the Air?* CD-ROM and study guide in your kit.

Impacts of Ozone on Animals (including humans) and Plants

Ground-level ozone is unstable and reacts chemically with plants, rubber, and the tissues of living creatures. Kids and elderly people are affected the most.

Ozone has the ability to irritate your lungs or break down your lung tissues. Ozone can trigger an asthma attack, and it can cause asthma attacks to be worse than usual. Even people who don't have asthma can have trouble breathing on days with high levels of ground-level ozone, especially people who spend a lot of time outdoors on those days.

Many plants that are sensitive to elevated ozone levels will show signs of visible injury. Conifers and broad-leaf trees, shrubs, lichens and mosses, domesticated crops and grasses all have member species susceptible to ozone injury. Ozone reacts with photosynthetically active cells on the upper surface of leaves typically causing an interveinal injury.

The injury resembles dot-like lesions that are called stipples. The coloration of the stipples will vary by species, ranging from red to purple to brown to black. Injured plants may also drop leaves early and produce fewer healthy seeds. The injury may also increase their susceptibility to other problems like insects and diseases.

It is possible to find a plant with no signs of ozone injury growing next to a severely injured plant of the same species. Not all plants growing in the same area show similar injury. Genetic differences, soil moisture differences and age can cause this difference.

For a detailed description of ozone injury to common milkweed, refer to “Evaluating Ozone Injury” under Part A of the Procedure, below.

The Big Picture

Air pollution harms plants, animals, humans, and entire ecosystems. By documenting visible symptoms on sensitive plants, scientists can determine how air pollution affects natural systems; much like the canaries warned miners of poor air quality in mines. Studies like this one will contribute to a statewide body of information about ozone formation and transport. Citizens and policy makers can then use this information to find ways to reduce air pollution.

Common Milkweed

Common milkweed (*Asclepias syriaca*) is the bioindicator plant for this study. Common milkweed is sensitive to ground-level ozone, and is found throughout Wisconsin.

For detailed information about common milkweed, please refer to the Milkweed Fact Sheet, The Anatomy of a Milkweed Plant sheet, or field guides such as the Peterson or Audubon Field Guides.

Monarch Butterfly

The monarch butterfly has a close ecological relationship with the milkweed plant. During this activity, students will note the presence of monarch caterpillars and butterflies on the plants they study.

The monarch’s survival is closely linked to the chemical defense system derived from the milkweed toxins and the nutrition supplied to the developing larvae. Studies like this one may help determine if ozone injury to milkweed leaves affects the larvae and life cycle of the monarch butterfly.

For detailed information about the monarch, please refer to the Monarch Butterfly Fact Sheet or field guides such as the Peterson or Audubon Field Guides.

Activity Overview:

1. Students will master an understanding of the difference between ground-level ozone and stratospheric ozone by reading the student summary.
2. Students will familiarize themselves with the identification of common milkweed by viewing the biomonitoring slide show and studying the milkweed drawing and photos.
3. Students will examine laminated samples of ozone-injured leaves with varying degrees of injury. They will become familiar with identifying ozone injury and distinguishing the injury from insect chewing and disease.
4. Students will establish a milkweed study plot.
5. Students will collect and press sample leaves, determine incidence of ozone injury (if any), and estimate the extent of the injury for each leaf.
6. Students will make some predictions about the incidence of ground-level ozone in the neighborhood of the study plot.

Materials:

1. Plant press (or book)
2. Hand lenses, at least one per group of 4-5 students
3. One fine-point, permanent soft-tip pen (e.g., *Sharpie* ®) per group of 4-5 students (used to write on collected leaves)
4. Kit supplies:
   - Laminated leaves (previously collected by teacher)
   - Milkweed photos and drawing
   - Biomonitoring PowerPoint presentation and notes
5. Student Worksheets (attached) Note: do not hand out Worksheet #1 until Part A, #3.

Procedure:

Part A: Understanding ozone, Identifying milkweed, Identifying ozone injury to milkweed

1. *Ground-level ozone and stratospheric ozone are the same chemical, but are found in different layers of the atmosphere and thus have different impacts on animals (including humans) and plants.*

Discuss the differences between stratospheric ozone and tropospheric ozone with the students, using the background information.

A clear understanding of the difference between the effects of these two types of ozone is essential to this activity.

2. *Milkweed plants have a unique morphology that can be used to identify them.*

Ask the students to carefully examine the drawing of common milkweed, and the pictures in the slide show and/or photos. Discuss the structure of the plant and ways to distinguish it from other plants.

3. *Ozone injury to milkweed plants can be identified by its distinctive appearance and pattern.*

A. Discuss with the students how to correctly identify ozone injury to milkweed leaves, using the summary below, called "Evaluating Ozone Injury."

B. Set up three lab stations. Divide the class into groups of 4-5 students, and organize them so that they rotate through the lab stations, one group at a time per station.

- At Station 1, place a laminated leaf showing ozone injury, and label it "Ozone Injury."
- At Station 2, place a laminated leaf showing other types of damage (insect chewing, disease, etc.), and label it "Other Damage." This leaf may also have some ozone injury, just make sure it shows another type of damage.
- At Station 3, place a leaf of your choosing and label it "Unidentified."

Students should rotate through the stations, using Student Worksheet #1 and a hand lens to examine the leaves. Each student should then circle the correct answer to the quiz at the bottom of Student Worksheet #1, and return to his/her seat.

Review the answer to the quiz, and recap the procedure for correctly identifying ozone injury. Do not collect the quizzes, because the students will use the information at the top of the sheet during the lab later on.

Evaluating Ozone Injury
Ozone injury on milkweed leaves is unique and relatively easy to diagnose. This injury typically results in sharply defined, small dot-like lesions (stipples) on the upper surface of the leaves. These lesions are observed ONLY on the upper leaf surface, and are black to dark

purple in color. If you turn the leaf over and the stipples show on the bottom, the damage is not ozone injury.

Veins are never affected, because ozone only reacts with photosynthetically active cells. If injury is severe it may produce an overall dark discoloration of the upper leaf surface as the lesions come together. In this case, you will need to use a hand lens to determine whether or not the lesions cross over the veins. If the damage affects the veins, it is not ozone injury.

Injury location on the leaves may vary considerably. In general, the location of ozone injury on a leaf is determined by the maturity of the leaf. Acute ozone injury tends to develop towards the tip of young leaves, in the center of fully-grown leaves, and at the base of older leaves. Leaves frequently exposed to ozone may exhibit injury symptoms all over the upper leaf surface.

Part B: Establishing a study plot, Collecting samples, Creating a student portfolio

Analysis of biomonitoring data gives evidence of air pollution.

1. Establishing/Selecting the Study Area

Select a study area that satisfies the four following criteria:

1. The plants should be at least 50 feet from any road to avoid the effects of vehicle exhaust, road salts and roadside cuttings. Abandoned fields, remote unmowed areas in parks or prairies are ideal sites. A milkweed study area can be established on a school site by transplanting sensitive plants to a sunny, protected site near the school.
2. Each study area should contain at least 20 milkweed stems. The stems should be within 100 feet of each other to minimize the influence of changing soil, topography or other site conditions.
3. Ten stems must be randomly chosen for study. Each stem should have 10 or more leaves. Leaves missing more than 10% of their surface area due to disease or insect chewing should not be evaluated.
4. Each milkweed should only have one main stem. Multiple stemmed/branched plants should be avoided.

The study area should be protected to minimize losses. Staking your plots and running string to the corner stakes may be required to prevent unintentional destruction. Study areas should be in secure areas, so the plants can be studied over the summer months. Note: Be sure to have the landowner’s permission if you are on private land.

2. Collecting/Pressing Leaf Samples and Evaluating Injury

Collection/evaluation should occur in late August or early to mid-September.

1. Divide the students into groups of 4 or 5. Assign each group an identification letter (A, B, etc.). Each student should record this identification letter at the top of his/her data sheet.

2. Each group should collect 10 sample specimens of injured and uninjured leaves – taking only ONE leaf from each of 10 plants - and hand them in with the completed data sheet. The leaves should represent high, intermediate, and low levels of ozone injury found on your site (if injury is not found, simply collect 10 leaves of different sizes and continue following the instructions). If the study plot is small, each group may share the same 10 leaves for analysis.
3. As each leaf is collected, students should follow the instructions on the student sheet for recording data about the plant and evaluating injury for the leaf collected.

To press and dry the leaves:

1. Each leaf should be cut from the stem during the site inspection and labeled by carefully writing the group number and plant number in small print directly on the leaf with a fine-point, permanent soft-tip pen (e.g., *Sharpie* ®). For example, a leaf collected by group A from plant 5 would be A5. This will help with later tracking of "which leaf was collected by which group from which plant."
2. The leaves should be pressed <u>immediately</u> upon being cut from the stem. Using a plant press works best, although students may also use a book. The press should be left in a well-ventilated room to enable the leaves to dry properly.
3. Drying will take 7-10 days.

Making a plant press
A simple press may be made by placing the leaves between sheets of newspaper and inserting the newspaper between two sheets of cardboard. Sandwich this between two pieces of wood or hardboard and close tightly using rope or weights.

3. <u>Creating a student portfolio:</u>

Each group should hand in the following:

1. A map showing study area locations. Include features near the site that would allow a person to find the site easily. (roads, buildings etc.)
2. Student Worksheets #2 and #3 (Data Sheet and Analysis).
3. Ten leaves, injured or uninjured, pressed and dried. (Note: Students may not find any ozone-injured leaves.)

Extensions/Modifications:

1. This activity can be done by a student or small group as an independent project, rather than an entire classroom activity.
2. If you teach summer school or summer camp, the best times to observe milkweed for ozone injury are late July and early August, in addition to the periods listed.
3. This activity may be done as an indoor activity only, for classrooms that may have difficulty arranging the field trip. If you plan to do it as an indoor activity, follow the instructions up to Part B of the Procedure. Skip Part B, and have students answer only questions 3,4, 7, and 8 on the Student Worksheet #3: Analysis.
4. WDNR has an ozone monitor available for classroom use. The monitor gives constant readings (in parts per billion) of ozone concentrations. This is a quantitative measure of ozone, and can be used to supplement the qualitative measure shown by the milkweed. Ground-level ozone is never completely absent from the troposphere. Ground-level ozone must reach certain levels in order to harm milkweed, so even if no ozone is detected via milkweed, the ozone monitor will probably detect small (harmless) amounts. Contactl Lindsay Haas (Lindsay.Haas@dnr.state.wi.us) to arrange to borrow the ozone monitor.
5. Ozone affects other plants in addition to common milkweed. If you would like to check out some other species, these are the plants to examine: blackberry, black cherry, big leaf aster, spreading dogbane, soybean, grape, tall or forest milkweed, and white ash. See *Other Species Considered Good Bioindicators*.

Milkweed Monitoring: Keeping a Finger on Nature's Pulse

Student Guide

Background:

Did you know that plants and animals can give us clues about pollution in our air, land, and water, before humans even notice that something is wrong? These sensitive species are called bioindicators. Even lichens (an unusual plant, made up of a blue-green algae and a fungus) and birds can tell us about air quality. In Wisconsin, common milkweed is a good plant to study because it is affected by ground-level ozone (smog) and it grows in a lot of areas.

Biomonitoring is the term scientists use to describe the use of plants, animals, or entire ecosystems to tell if our environment is polluted. Biomonitoring has been used by biologists and scientists to give us information about our surroundings for a long time. Here are three examples.

Canary in a coal mine

Have you ever heard the phrase "Canary in a coal mine?" In the 1800s few jobs were harder or more dangerous than working as an underground coal miner. Over the years, thousands of men, women, and even children were killed in mine accidents. One common cause of the accidents was a build-up of dangerous gases like methane and carbon monoxide in the mine shafts. Large amounts of these gases could lead to violent explosions. Methane and carbon monoxide are colorless and odorless. The miners of the 1800s didn't have the special equipment scientists have today to measure chemicals in the air, so it was impossible to tell if the gases were building up to dangerous levels. Miners started to use canaries to test the air quality in the mines. Canaries are very sensitive to carbon monoxide. The canaries would chirp and sing all day long. But, if the carbon monoxide levels got too high, the canaries would have trouble breathing, and maybe even die. When the canaries were no longer singing, miners would know the levels of the gases were too high. They would leave the mine quickly to avoid being caught in an explosion. Miners used the canaries as a warning system for dangerous mine conditions.

Eagle drop

Eagle populations suddenly dropped in the 1960's, leading to the discovery that certain pesticides could build up in the food chain and change natural systems. Eagles began to lay very thin-shelled eggs. The eggs often broke, or if they hatched, the chicks were often weak and died within a few hours or days. It looked as if eagles might become extinct. Scientists studying this problem began to wonder if the chemicals that were harming eagles might be able to harm humans and other types of animals, too. The scientists told their concerns to the government, and new laws were made to stop the use of many harmful chemicals. After a few years, eagle populations began to improve.

Milkweed polka dots

One way we can learn more about air pollution is to study a type of plant that is very sensitive to air pollution. Common milkweed is one of these plants. It shows signs of injury from ozone by showing stipples, or dark polka dots, all over the leaves; losing leaves; turning strange colors; or not growing very big. Your class will conduct a study of milkweed to find out if there is ground-level ozone pollution in your area.

Activity: Milkweed Check-Up

You can be an environmental doctor and conduct a simple ozone injury check-up on milkweed. Ozone injury on milkweed can be seen on the leaves. This injury is unique and is pretty easy to identify.

As a plant doctor, you can look for these symptoms: sharply defined, small dot-like lesions, called stipples, on the top (upper surface) of the leaves. These markings appear only on the top of the leaf and are black to dark purple in color. If the ozone effects are severe you'll see a large dark area on the upper leaf surface as the markings blend together.

Ozone injury can look different on leaves of different ages. Minor ozone injury on young leaves can be found on the tip of the leaf. It is found in the center of fully grown leaves and at the base of older leaves. Leaves that are exposed to lots of ozone may show injury symptoms all over the upper leaf surface.

Procedure

Equipment: Data Sheet, pencil, fine-point permanent marker, clipboard, extra paper

1. Draw a map showing the location of the study area on the back of the data sheet. Show features near the site like roads, trails, signs and buildings that would allow a person to find the site.
2. Complete the information at the top of the data sheet. Your teacher will give you a "group ID letter" for your group.
3. Fill out the data sheet as follows:
    a. Select your first milkweed plant. Make sure it has only one main stem, and that it has at least 10 mature leaves (mature leaf = 5-10 centimeters in length).
    b. Measure and record the height of the stem from base to top in centimeters.
    c. Count and record the total number of leaves on the plant.
    d. Examine the leaves carefully with a hand lens. Is there ozone injury? Use the hints at the top of Student Sheet #1 if you need help deciding. Count and record the number of leaves showing ozone injury on the plant (there may not be any). Evaluate only mature leaves of at least 5-10 centimeters in length.

e. Calculate the percentage (%) of ozone injured leaves per plant (use the total # of leaves and the total # of ozone injured leaves for this calculation).

f. Collect and press a sample leaf from each plant. Try to collect an ozone injured leaf. If no leaves are ozone injured, select any leaf. Write each leaf's ID # directly on it in small letters with your permanent soft-tip pen. The leaf ID # is your group letter plus the Plant ID#. For example, if your Group ID letter is **A**, and the leaf came from plant #**5**, then the leaf ID # is **A5**. Your teacher will collect the leaves at the end of the lab for pressing and drying.

g. For each leaf collected, record what percentage of the leaf area that you estimate is covered by ozone injury (stipples), using the code below. Try to select leaves that show a variety of ozone injury. Many plants may have no ozone injury. Avoid leaves that are more than 10% missing due to insect chewing, disease or other non-ozone injury. If you're having trouble, ask your teacher to show you the laminated leaves or photos again.

    <u>Code for recording % of leaf area injured</u>:

    0 = no visible signs of injury

    1 = very light to light (1-15%)

    2 = moderate to moderately heavy (16-50%)

    3 = heavy (> 50%)

h. When you finish evaluating the leaf, put it inside a book or plant press to protect it.

i. Record the presence of monarch butterflies and the developmental stage (the number of larvae, chrysalis or butterflies on each plant).

j. Record the number of seed pods per plant.

k. Feel free to record observations you find interesting at the bottom portion of the data sheet, labeled "Field Notes." For example record the size of leaves, whether there are aphids, other types of damage, the health of the plants, weather, etc. Plants heavily infested with aphids may develop a black sooty mold on the upper surface of the lower leaves. This makes finding ozone injury difficult.

l. Proceed to the next selected plant and follow this procedure until 10 plants have been evaluated. Each of the plants you evaluate should be 5 feet from each other, if possible.

m. Once you've finished evaluating 10 plants, complete the field analysis at the bottom of the chart, as it applies to all the plants you evaluated. Helpful information: Younger leaves are usually toward the top of the plant, older ones near the bottom. Younger leaves are a lighter green color and are often smaller. They look 'new.'

4. Complete the Student Worksheet #3: Analysis. Your teacher will collect this at the end of the lab.

Glossary:

Biomonitor: a plant or animal that serves as an indicator of air or water quality.

Chloroflourocarbons: a compound made up of chlorine, fluorine, and carbon atoms. This compound was commonly used for many years in refrigerators and air conditioners. Now banned in most countries. Destroys stratospheric ozone.

Chrysalis: a pupa of a butterfly.

Fossil Fuel: fossil fuels (coal, oil, and natural gas) make up the biggest part of our sources of energy. Fossil fuels were formed over millions of years from the remains of dinosaurs and prehistoric plants. When fossil fuels are burned, they produce pollution.

Ground-level Ozone: (also called tropospheric ozone) ozone that is formed in the troposphere when chemicals (NOx and VOCs) produced mainly by cars, power plants, factories are baked in the hot sun. Ground-level ozone makes it hard for us to breathe, and is harmful to the leaves of plants and trees.

Migration: movement of animals from one seasonal location to another.

Nitrogen Oxides (NOx): nitrogen and oxygen combine to form different varieties of this compound. Each variety has a different number of oxygen atoms that have joined together with a nitrogen atom. (The 'x' at the end of NOx can stand for 1, 2, 3 or more oxygen atoms. That's why it's an 'x' and not a number.) When NOx is baked with VOCs in the hot summer sun, ground-level ozone is created.

Nonattainment Areas: regions of the United States where air pollution (including ground-level ozone) has been measured at unhealthy levels. These regions are required by the Clean Air Act to reduce the amount of pollution in order to protect the health of people, plants and animals. Southeastern Wisconsin is a nonattainment area.

Ozone: a very reactive form of oxygen, O_3.

Stratosphere: the layer of the atmosphere that is found 6 to 30 miles above the ground. It contains stratospheric ozone.

Stratospheric Ozone: ozone found in the stratosphere. Stratospheric ozone is the same chemical as ground-level ozone, but because it is high above the Earth, stratospheric ozone is helpful, not harmful. It protects us by blocking the most harmful rays of the sun - ultraviolet rays - which can cause skin cancer.

Troposphere: the layer of the atmosphere reaching from the ground up to about 6 miles.

Ultraviolet, or UV: Light rays can be divided into three main groups: visible light, ultraviolet light, and infrared light. Visible light is what humans can see. We can't see ultraviolet light and infrared light, but they are present in sunlight, and can cause sunburns or health problems like skin cancer and cataracts.

Volatile Organic Compound (VOC): In chemistry, organic means something containing carbon. Volatile means 'easily evaporated'. It can also mean explosive. And a compound is something that is made up of two or more other elements. So, a volatile organic compound is a carbon substance that is made up of two or more elements, is easily evaporated, and may be explosive.

Student Worksheet #1: Identifying Ozone Injury

About ozone injury:

- Ozone injury to milkweed shows up as 'stipples', which are little black or dark-colored spots on the top of the leaf.
- Ozone <u>only</u> affects the tops of the leaves, so check the bottoms, too. If you see stipples on the bottom, then it's not ozone injury.
- It is NEVER found on a vein of the leaf. Remember, it affects the cells that are making food. Veins in plants are nutrient-carrying systems, so it wouldn't affect them. So if you see stipples on the veins, then it's not ozone injury.
- The stipples don't have to cover the entire top of the leaf in order to be ozone injury, but they might.
- If you are unsure, ask for help.

Instructions:

1. First, study the leaf marked "Ozone injury" to get an idea of what it looks like. Carefully scan the top surface. Pick it up and look at the bottom. Use the hand lens to look very closely at the leaf.

2. Second, study the leaf marked "Other damage" to get an idea of what other types of damage look like. These could be fungus, insect chewing, or some type of disease. Note that these types of damage <u>do</u> cross over the veins and appear on the bottom. Careful – the leaf may have ozone injury in addition to the other types of damage.

3. Last, investigate the unidentified leaf. It may or may not have ozone injury. See if you can determine whether or not the leaf has been injured by ozone. Record your answer below.

Quiz:

Circle the correct answer for the unidentified leaf:

Other damage   Ozone injury   Both

Student Worksheet #2: Data Sheet

Group ID letter: _____ Names of students in group: Date(s):	Plot Location Describe location: Address: City/Town: County:	Weather:

Plant ID #	Plant Height (in cm)	Total # Leaves	# of Injured Leaves per plant	% Injured Leaves on Plant	% Leaf Area Injured (use code #s 0-3)	Monarch Butterfly (Stage and number)	# of Seed Pods
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

Field Notes:

Student Worksheet #3: Analysis

1. What level of injury did you find?

2. If you found no ozone injury, what might this mean? How can you verify your results?

3. What is a bioindicator? How can using milkweed and other plants as bioindicators help us keep the air clean?

4. What are the advantages or disadvantages to using biomonitoring projects?

5. Compare your findings with those of the local air quality monitoring agency. Are they similar? Different? What might cause the results to be different?

6. Compare your findings with other schools inside and outside your geographic area. What similarities are there? What differences are there?

7. What causes ozone to be created in the lower atmosphere? What problems can it cause?

8. What can an individual do to reduce ozone pollution? What can YOU do?