

# Milkweed Monitoring

## Background

This project uses milkweed plants, which grow in many places around the world, to look for signs of air pollution. Specifically, milkweed plants are damaged by a type of air pollution called ground-level ozone. This pollutant is the same chemical as stratospheric ozone (the ozone that protects us from the sun's harmful ultraviolet rays), but it is found in the troposphere - our breathing space. Ground-level ozone is a volatile chemical, so it reacts with things it comes into contact with - like plant tissue, lung tissue, and mucus membranes (eyes and nose). Ground-level ozone can cause respiratory problems and can trigger an asthma attack.

Ozone forms at ground-level when pollutants from vehicles, power plants, and chemicals like fumes from paint thinner, bake in the hot sun. The primary chemical families that these pollutants fit into are NO<sub>x</sub> (Nitrogen Oxides) and VOCs (Volatile Organic Compounds). Ground-level ozone needs hot weather and sunlight in order to form. Before you do this study, you'll need to determine when ground-level ozone might be present in your area. Generally that's mid- to late summer; for example, in the U.S., most ozone damage to plants occurs from June to September.

Ground-level ozone damages many types of plants. This study uses common milkweed because it is relatively plentiful and shows distinct signs of damage and injury. When the milkweed plant is involved in photosynthesis, it takes up carbon dioxide from the air. It also takes in anything else that's in the air, so if ground-level ozone is present, then it is 'breathed' in by the plant. Ground-level ozone attacks individual photosynthetic cells on the tops of the leaves, leaving small black dots - called stipples - on the top surfaces of the leaves.

### Ozone Injury ID - Checklist:

Other types of damage to milkweed plants can sometimes resemble ground-level ozone injury. It's important to know how to accurately identify the injury. There are four major characteristics:

1. Ground-level ozone injury looks like small black dots on the top of the leaf. It's not brown or red or purple - it's black.
2. Ozone only affects the tops of the leaves, so check the bottoms, too. If you see stipples on the bottom, then it's not ozone damage.
3. It is NEVER found on a vein of the leaf. Remember, it affects only photosynthetic cells - the cells that are making food. Veins in plants are vascular (water-carrying) systems, so it wouldn't affect them. So if you see stipples on the veins, then it's not ozone damage.
4. The stipples don't have to cover the entire top of the leaf in order to be ozone damage, but they might.

Studying plants to determine the health of an ecosystem is called biomonitoring. Milkweed is a bioindicator plant, because it reacts to pollutants at levels below those that affect human health.

To see some photos of ozone injury to milkweed leaves, and learn more about biomonitoring, [click here](#).

Are you ready? If you...

- ✓✓ have read the background information above, and
- ✓✓ have looked at the [photos](#) of ozone injury to milkweed, and
- ✓✓ can identify a milkweed plant, and
- ✓✓ know what ozone injury to milkweed leaves looks like, and
- ✓✓ you have milkweed nearby... THEN...

You're ready! The study procedure is outlined below, and the data-reporting sheet is the last page of this file. Print this file and head out into the field.

## Procedure

Equipment: data sheet, pencil, fine-point permanent marker, clipboard, extra paper, plant press or book

1. Select a study site that satisfies the following criteria:
  - ~~///~~ 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.
  - ~~///~~ 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.
  - ~~///~~ 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.
  - ~~///~~ 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. 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. Don't forget to include an arrow showing which way is **north** on your map.
3. Complete the information at the top of the data sheet.
4. 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 or magnifying glass. Is there **ozone injury**? The plant may have other damage like insect chewing or a virus, but you're looking specifically for ozone injury. Use the checklist the background information on page one of this study if you need help deciding whether or not what you see is ozone injury. 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. Using your permanent marker - not your pencil - label each leaf according to its corresponding plant ID number found in the first column of your data sheet.
  - g. For each leaf collected, record the leaf's growing location on the stem (top, middle, or bottom third).
  - h. 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.

Code for recording % of leaf area injured:

    - 0 = no visible signs of injury
    - 1 = very light to light (1-15%)
    - 2 = moderate to moderately heavy (16-50%)
    - 3 = heavy (> 50%)
  - i. If the leaf you collected has ozone injury, record the location of the ozone injury by coloring it on the leaf drawing on the chart. The drawing shows the top of the leaf, with the leaf stem to the left.
  - j. For the leaf you collected, record your estimated percentage of the total leaf area affected by non-ozone injury (discoloration, disease, insect chewing, etc.).
  - k. When you finish evaluating the leaf, put it inside a book or plant press to protect it.

- l. Record the presence of monarch butterflies and the developmental stage (the number of larvae, chrysalis or butterflies on each plant).
- m. Record the number of seed pods per plant.
- n. 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 and diagnosing ozone injury difficult.
- o. 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.

## Pressing and Drying the Sample Leaves

Place each leaf in a plant press or between the pages of a book (each leaf should be separated from the others by several pages). Drying usually takes 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.

## Data Analysis

Now that you've collected the data, it's time to analyze it and tell us what you think about it. Use the Data Analysis Questions on the next page to do that.

## Submitting Data

Please follow these instructions carefully to ensure prompt, accurate posting of your data.

1. Photocopy the following items:
  - ☞ your completed Data Sheet, and
  - ☞ the Data Analysis Sheet, and
  - ☞ the site map you drew.

2. Mail:
  - ☞ the copies of the three above items, and
  - ☞ three sample leaves

To:

Easy Breathers: The Lab  
CE/6, Box 7921  
Wisconsin DNR  
Madison, WI 53707-7921











3. Please note: we cannot mail your data back to you, so please copy it before you send it. If you need us to return it and/or your leaves, you must enclose a self-addressed, stamped envelope.

## Data 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 (if possible). What similarities are there? What differences are there?
7. What can an individual do to reduce air pollution? What can YOU do?

## Data Sheet

<b>Name:</b>  <b>Grade Level:</b> _____  <b>Date(s):</b>	<b>Plot Location:</b> (Describe location below and then draw a map on the back of this data sheet)  <b>City/Town:</b> <b>Country:</b>	<b>Describe today's weather:</b>  <b>Growing season weather:</b>
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					THESE COLUMNS REFER TO THE LEAF YOU COLLECT FROM EACH PLANT					
Plant ID #	Plant Height (in cm)	Total # of Leaves	Number of Ozone Injured Leaves	% Ozone Injured Leaves	Leaf Location Code: 1=top third 2=middle third 3=bottom third	% Leaf Area Injured by Ozone: 0 = no injury, 1 = ? 15 %, 2 = 16-50 %, 3 = >50 %	Location of Ozone Injury on Leaf? Color in the area on the leaf below.	% Leaf area Affected by Other Injury (discoloration, diseased or insect chewing)	Monarch Butterfly (stage and number)	# of Seed Pods
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

**Field Notes:**