NATURAL RESOURCES PRESERVATION BRIEF #10

MILKWEED - OZONE STUDY

Introduction

Extensive areas of the eastern United States experience significant levels of photochemical smog of which ozone is a major constituent. Ozone is highly toxic to vegetation, and injury to plants is suspected to be occurring in many National Park units. It is imperative to the responsibility of the NPS that it be determined whether ambient concentrations of ozone are causing vegetational injury in park units, and if they are, how extensive the injury is. To answer these questions, and so that the NPS can take appropriate action to protect park resources, it has been decided by the AWQD to use plants as biomonitors of air pollution by conducting a project with a known sensitive species in the parks where it occurs.

Common milkweed, Asclepias syriaca, has been selected because it is a very common species and has a fairly broad range of occurrence within the eastern U.S. Common milkweed is particularly sensitive to ozone, an unstable pollutant gas which causes pigmented spots (st ippling) to appear on the upper leaf surface due to the collapse of cells beneath the epidermis. This study, therefore, will benefit the NPS by monitoring air quality and its effects across five NPS regions.

Methods

General

Each participating park will identify areas where common milkweed is growing. If the species was observed last year it will probably be found in the same vicinity since it is propogated from underground rhizomes as well as seed. Each park should find a number of sites (1 plot at each site) which satisfy the following criteria. The number of plots per park is dependent on the abundance and distribution of \underline{A} . syriaca within the park and the size of the park. The number of plots per park should be chosen such that:

- 1. The park is adequately represented throughout the range of \underline{A} . syriaca in the park. Ten (10) plots per park are adequate for most parks. If a park is small in area then it may not be possible to find 10 plots; in this case a lesser number will suffice.
- 2. The plots themselves should be as well spaced as possible within the park (including a range of elevations if possible).
- 3. The plots should not be located immediately adjacent to any roads. They should be at least 30-50 meters from the road to avoid confounding effects of exhaust pollutants.
- 4. Each plot should contain no less than 10 individual plants. There are no limitations on the size of the plot at each site. NOTE: The plants should not be spaced so far apart that the environment changes appreciably, as plant growth may not be constant under variable conditions. Generally, all 10 plants should be within 30-50 meters of each other.

Plot Establishment

The plots in each park should then be numbered 1-10 and located on a topographic map. From each plot a minimum of 10 individual plants should be selected. Each plant should have 10 to 16 leaves more than 65% fully expanded or more than 10 cm in length. Each plant and leaf should be marked in some way, e.g., using paper tags on string (as inconspicuously as possible) to number the plants and waterproof ink, colored yarn, etc. to mark the petiole of each evaluated leaf or pair of leaves. These numbers should be retained for the duration of the study. Each plot should be afforded some kind of protection (e.g., from mowing, etc.) to minimize losses. Staking the plots and running string to the corner plot stakes may suffice to prevent unintentional plot destruction. The plots should not be made permanent, but should be relocatable in future years if the project is continued.

Two ozone injury evaluations are required. The first evaluation should occur between June 15 and June 30. The second evaluation must be between August 15 and August 30. Evaluation of the plots <u>must</u> be performed during these time periods to allow for comparisons of injury between parks.

Visible Foliar Injury Symptoms From Exposure To Ozone.

Ozone produces a type of injury on milkweek leaves that is unique and easy to diagnose. This injury typically results in sharply defined, small dot-like lesions which are observed only on the <u>upper leaf surface</u>. These lesions are frequently black-purple in color. The veins are usually not affected. Pigment formation may produce an overall dark discoloration of the upper leaf surface when lesions are dense and coalesce. Please ignore all other leaf injury symptoms in your evaluations.

The position of injury on the leaf may vary. In general, the location of ozone injury on a leaf is determined by the developmental sequence of tissue maturation in the leaf. Leaf cells that are fully differentiated, have developed intercellular spaces but do not yet have suberized cell walls, are the most sensitive to ozone injury. Consequently acute ozone injury tends to develop towards the tip of younger leaves, in the center of older ones, and at the base of the oldest affected leaves. Foliage frequently exposed to ozone may exhibit injury symptoms anywhere on the upper leaf surface. Leaves ranging from about 65-95% of their full size tend to be the most sensitive to ozone injury.

Procedures For Plot Evaluation

For each plant in each plot:

- Measure the individual heights of the plants from base to apex in centimeters.
- 2. Count the number of leaves showing ozone injury.
- 3. Count the total number of leaves on the plant. Do not include apical leaves which are not fully expanded, i.e., those which are still in a vertical position and have not oriented their upper surfaces to the sun. In general, evaluate only leaves that are at least 65% fully expanded.

- 4. Estimate the percentage of the total leaf area of each leaf that has ozone injury. If the number of mature leaves on the plant exceeds 10 (which usually will occur by the second sampling date) then enter data for the uppermost 10 mature leaves only.
- 5. Record any presence of the monarch butterfly (larvae, chrysalis, butterfly) on each plant.
- 6. Record the number of seed pods per plant.

From the data collected the investigator should then calculate the means and standard deviations and enter these on the data sheet in the spaces provided.

Monarch Butterfly

The monarch butterfly (<u>Danaus plexippus</u> Linn.) has a close ecological relationship with the milkweed plant. Eggs are deposited on the plant and the developing larvae feed on the leaves, ingesting cardiac toxins in the leaves which render the mature butterflies unpalatable to predators.

The monarch is one of the few North American butterflies that migrates to South America for the winter. Several generations are produced each winter in South America before the monarch returns to its summer breeding home in North America. The returning monarchs then produce two generations before returning to South America in the fall to repeat the cycle.

Monarch survival is tied closely to the chemical defense system derived from the milkweed toxins and to the nutrition supplies to the developing larvae from the leaves of the plant. Asclepias syriaca is an important species and a common host for the monarch. The relatively high susceptibility of common milkweed to ozone injury and the prevalence of ozone injury on milkweed plants throughout the eastern United States suggests the hypotheses that ozone injury on milkweed leaves may affect the life cycle of the monarch butterfly. In order to determine if monarchs are feeding on our milkweeds, we would like you to record the presence of any developmental stage of the monarch butterfly if you see it on any plants in the plots.

The Natural Resources Management Staff