

STANDARDIZED PROCEDURES FOR ESTABLISHING PLOTS
AND EVALUATING POLLUTION INJURY
TO COMMON MILKWEED IN THE
EASTERN UNITED STATES

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Procedures for Evaluating the Geographic Extent of Air Pollution Injury to Common Milkweed (*Asclepias syriaca* L.) in National Park Service Units in the Eastern United States

Objective

The objectives of these procedures are to employ standard methods for determining the geographic extent of air pollution (ozone injury) to Common Milkweed in the Eastern United States and to determine the relative severity of ozone injury between National Park Service units during the 1984 air pollution season.

Methods

Each designated park will identify areas where Common Milkweed is growing. If the species was observed during previous years it will probably be found in the same vicinity since it grows from underground rhizomes.

Each park should find 10 sites (1 plot at each site) which satisfy the following criteria:

1. The sites themselves should be as well spaced as possible within the park (including a range of elevation if possible).
2. The sites should not be located immediately adjacent to any roads. They should be at least 30 meters from the road to avoid exaggerated effects of exhaust pollutants.
3. Each site should contain no less than 10 individual plants (aim for at least 15). There are no size limitations at each site for the plants to grow within. IMPORTANT: The plants should not be spaced so far apart that the environment changes appreciably, as plant growth may not be constant under variable conditions.
4. The sites should not be located in highly forested areas as shading changes the plants' sensitivity to ozone and dense canopies influence ozone concentrations.

If the sites satisfy these criteria then they may be considered suitable.

Note: If a park is small in area then it may not be possible to find 10 plots; in this case a lesser number may suffice but should be no less than 7.

The plots in each park should then be numbered 1-10 and located on a 1:62,500 topographic map. From each plot a maximum of 15 (preferable) but no less than 10 individual plants should be selected. If possible they should be approximately of equal height (and therefore age) and have 10 to 16 leaves present. Each individual should be marked in some way, for instance using aluminum tags as inconspicuously as possible and assigned a number from 1 to 10-15. This number should be retained for the duration of the investigation. Each plot should be afforded some kind of protection (e.g., from mowing etc.) to minimize losses.

Three repetitions of sampling should be done, between the beginning of July and the end of August. The best times would be the first week in July, the last week in July, and the third week in August, but any time will do providing that there is adequate spacing between sample dates.

As this is a one season study, the plots should not be made permanent.

The investigator will be required to return to the Denver office:

Sample Period 1

1. 1:62,500 topographic maps showing plot locations.
2. Pressed plants showing ozone injury, and relevant related data (see later notes).
3. Data sheets for all 10 plots.

Sample Periods 2 & 3

Data sheets for all 10 plots for each of the two sampling dates.

IMPORTANT: Data from period 3 should reach the Denver office no later than Friday, August 31st.

Visible foliar injury symptoms due to ozone.

Localized thickening and pigmentation of the cell walls results in sharply defined, small dot-like lesions which are observed primarily on the upper leaf surface. These may be dark brown, black, purple or red. The veins are not usually affected. Pigment formation may produce an overall discoloration of the upper leaf surface when lesions are dense. Small necrotic spots or more general upper surface bleaching may occur (chlorosis). Also, light gray, milk-white, or tan color may result due to air spaces where cells have collapsed. The position of injury on the leaf may vary: It tends to develop towards the tip of younger leaves, in the center of older ones, and over the whole, or base, of the oldest affected leaves. Leaves ranging from about 65-95% of their full size tend to be the most sensitive to ozone injury.

PROCEDURE FOR SAMPLING.

For each plant in each plot (also see explanation of data sheet):

1. 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.
4. *Estimate the percentage injury to each leaf.
5. Record type(s) of injury found on the plant due to ozone.
6. Record any other type(s) of injury.

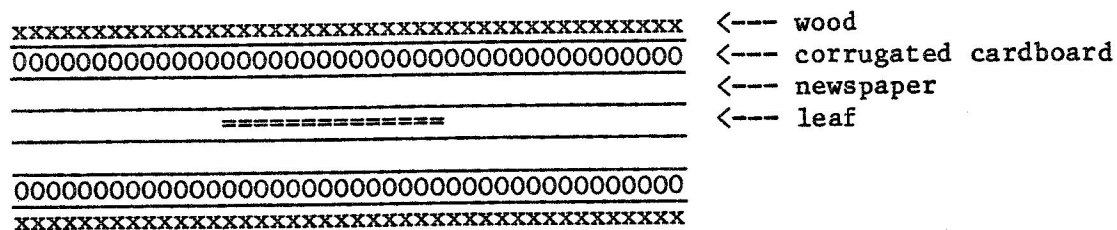
* If the number of leaves on the plant exceeds 20 (which may occur by the 3rd sampling date) then enter data for the uppermost 20 leaves (not including

apical leaves which have not fully expanded i.e., those which are still in a vertical position and have not oriented their upper surfaces to the sun, e.g. see Figure 1. in which the top 5 leaves would not be included).

From the data collected the investigator may then calculate the means and standard deviations and enter these on the data sheet in the spaces provided. (This is done easily using a scientific calculator.)

Pressed leaves should be prepared to illustrate the ozone injury. The leaves removed for pressing should be taken from similar nearby plants, not from those in the investigation. They should be representative of the injury found in the site e.g., include the greatest and the least levels of injury plus some intermediate levels. Five (5) leaves per plot will probably be sufficient. Pressed leaves and data relating to those individual leaves should be returned to the Denver office after the first period of sampling i.e., data for these leaves will only consist of percentages, injury symptoms and other injury types as explained. This data should be included in the package containing the pressed leaves. After pressing, the specimens should be delivered flat and should be unmounted. The best method to prepare pressed leaves is to place them in a plant press (with an appropriate label for identification), but if there is not one available then a simple press may be made by placing the leaves (separately and with identification), between sheets of newspaper and putting this between 2 sheets of corrugated cardboard. Sandwich this between 2 pieces of wood or hardboard and close as tightly as possible using rope or weights. The press should be left in a well ventilated room to allow the leaves to dry out (this will only take a few days). For mailing, the leaves should be removed from the press, and placed between sheets of wax paper and contained in a stiffened envelope.

Diagram of press



If there are any questions relating to the procedures, do not hesitate to contact us (numbers given on the front of this manual).

gynostegium; further elaboration of the filaments produces hoods in *Asclepias*; in our species the pollen is borne in pollinia, each pollinium composed of two pollen masses from adjacent anthers; stigma 1, ovaries 2, ovules numerous. Follicle linear to lance-ovoid. Seeds usually comose.

PERIPLOCA GRAECA L., an occasional escape from cultivation, is listed in our area by some authors but no specimens or citations have been seen to confirm its range in the Carolinas.

- | | |
|---|-----------------------|
| Stem erect or spreading, not a vine | 1. <i>Asclepias</i> . |
| Stem climbing or trailing. | |
| Petals white, or tinged with green or rose and the leaves linear; | 2. <i>Cynanchum</i> . |
| follicles smooth | |
| Petals not white and the leaves never linear; follicles muricate | 3. <i>Matelea</i> . |
| or smooth | |

1. *ASCLEPIAS* L. MILKWEED

Perennial herbs, usually with milky sap. Stems erect, spreading or decumbent, usually simple and often solitary. Leaves opposite to subopposite, or sometimes whorled, rarely alternate. Corolla lobes reflexed, rarely erect or spreading; the filaments elaborated into five hoods forming a corona around the gynostegium, corona horns present in most of our species.

I. *Flowering Specimens*

- | | |
|--|------------------------------|
| Petals predominately white or green. | |
| Leaves linear, 10X or more as long as wide. | |
| Leaves whorled | 3. <i>A. verticillata</i> . |
| Leaves opposite or subopposite. | |
| Corona 3-8 mm broad; umbel solitary | 14. <i>A. michauxii</i> . |
| Corona 2-3 mm broad; umbels 2-5 | 18. <i>A. longifolia</i> . |
| Leaves lanceolate to ovate, less than 10X as long as wide. | |
| Gynostegium elevated 4 mm or more above hoods; plants usually less than 3 dm tall | 20. <i>A. pedicellata</i> . |
| Gynostegium not elevated above hoods; plants usually more than 3 dm tall. | |
| Inflorescence sessile or subsessile. | |
| Corona 2-3 mm broad | 19. <i>A. viridiflora</i> . |
| Corona 5-9 mm broad | |
| Horns longer than hoods, exerted apically; stem and leaves lightly tomentose | 15. <i>A. tomentosa</i> . |
| Horns shorter than hoods; exerted laterally; stem and leaves densely tomentose | 16. <i>A. obovata</i> . |
| Inflorescence pedunculate. | |
| Longest pedicels 3-6 cm long, often lax. | |
| Lower surface of leaf glabrous or essentially so; horns 1.5-2X as long as hoods | 7. <i>A. exaltata</i> . |
| Lower surface of leaf tomentose; horns equaling the hoods | 10. <i>A. suriaca</i> . |
| Longest pedicels 0.5-2.5 cm long, may be reflexed, rarely lax. | |
| Corona 2-3 mm broad; umbel 2-3 cm in diam.; horns longer than hoods | 2. <i>A. perennis</i> . |
| Corona 4-8 mm broad; umbel 3-6 cm in diam.; horns shorter than hoods | |
| Four largest leaves appearing whorled; corolla lobes less than 6 mm long | 9. <i>A. quadrifolia</i> . |
| Leaves all opposite; corolla lobes more than 6 mm long | 12. <i>A. variegata</i> . |
| Petals predominately rose, purple, or red to yellow | |
| Leaves alternate to subopposite, or some appearing whorled | |
| Leaves more than 12, alternate to subopposite; flowers red to yellow; sap not milky | 4. <i>A. tuberosa</i> . |
| Leaves 4-10, the 4 largest appearing whorled, or rarely subopposite; flowers pink; sap milky | 9. <i>A. quadrifolia</i> . |
| Leaves linear to narrowly lanceolate, 10X or more as long as wide. | |
| Corona 4-5 mm broad; leaves linear; follicles on erect pedicels | 17. <i>A. cinerea</i> . |
| Corona 7-10 mm broad; leaves lanceolate; follicles on deflexed pedicels | 6. <i>A. lanceolata</i> . |
| Leaves lanceolate to ovate, 6X or less as long as wide. | |
| Leaves sessile, auriculate. | |
| Plant erect; inflorescence usually solitary and terminal; corona 5-8 mm broad | 8. <i>A. amplexicaulis</i> . |
| Plant spreading-ascending; inflorescences 2-5, from upper nodes; corona 3-5 mm broad | 11. <i>A. humistrata</i> . |
| Leaves petiolate to subsessile, not auriculate. | |

- | | |
|---|------------------------------|
| Leaves glabrous beneath, or pubescent along veins. | 5. <i>A. rubra</i> . |
| Leaves subsessile, lanceolate; petals rose red | |
| Leaves petiolate, ovate to ovate-lanceolate; petals pink to white | 9. <i>A. quadrifolia</i> . |
| Leaves pubescent beneath. | |
| Hood margin with a single median triangular tooth; follicles muricate | 10. <i>A. syriaca</i> . |
| Hood margin without a median tooth; follicles smooth. | |
| Hoods 5-7 mm long; leaves ovate, 4-8 cm wide | 13. <i>A. purpurascens</i> . |
| Hoods 3-4 mm long; leaves lanceolate, usually 1-3 cm wide | 1. <i>A. incarnata</i> . |

II. *Fruiting Specimens*

- | | |
|--|------------------------------|
| Leaves alternate to subopposite or whorled (only the four largest appearing whorled in #4) | 4. <i>A. tuberosa</i> . |
| Leaves alternate; sap not milky | |
| Leaves subopposite or whorled. | |
| Leaves whorled, or appearing so. | 3. <i>A. verticillata</i> . |
| Leaves linear; follicle 4.5-8.5 cm long | 9. <i>A. quadrifolia</i> . |
| Leaves ovate to elliptic; follicle 8-11 cm long | 4. <i>A. tuberosa</i> . |
| Leaves subopposite. | |
| Leaves pilose or hispid, rarely linear | 14. <i>A. michauxii</i> . |
| Leaves glabrous or weakly pubescent, linear. | 18. <i>A. longifolia</i> . |
| Follicle terminal | |
| Follicle axillary, from an upper node | 2. <i>A. perennis</i> . |
| Leaves opposite. | |
| Follicle pendulous | 10. <i>A. suriaca</i> . |
| Follicle erect | |
| Follicle smooth | |
| Leaves glabrous beneath. | |
| Leaves linear. | 17. <i>A. cinerea</i> . |
| Leaves 1-2 mm wide | |
| Leaves 2-7 mm wide | 14. <i>A. michauxii</i> . |
| Follicle terminal | 18. <i>A. longifolia</i> . |
| Follicle axillary from an upper node | |
| Leaves not linear | |
| Leaves ovate to elliptic, auriculate. | 8. <i>A. amplexicaulis</i> . |
| Stems erect; follicles from solitary terminal inflorescence. | |
| Stems spreading-ascending; follicles from axillary inflorescences at upper 2-5 nodes | 11. <i>A. humistrata</i> . |
| Leaves lanceolate or lance-ovate, not auriculate. | 6. <i>A. lanceolata</i> . |
| Follicle 6-10 cm long; plant of cp. | 7. <i>A. exaltata</i> . |
| Follicle 12-14 cm long; plant of mts. | |
| Leaves variously pubescent beneath. | |
| Leaves linear or lanceolate. | 20. <i>A. pedicellata</i> . |
| Leaves linear; plant 1-3 dm tall | |
| Leaves lanceolate; plant usually more than 3 dm tall. | 19. <i>A. viridiflora</i> . |
| Leaves coriaceous; plant not of moist areas | |
| Leaves not coriaceous; plant of marshes, bogs or wet pastures. | |
| Follicle 6-10 cm long; pedicel erect; leaves pilose-hispid beneath; plant strongly branched | 1. <i>A. incarnata</i> . |
| Follicle 9-12 cm long; pedicel deflexed; leaves hispid along margin; plant weakly, if at all, branched | 5. <i>A. rubra</i> . |
| Leaves ovate to elliptic. | |
| Leaves tomentose beneath. | 15. <i>A. tomentosa</i> . |
| Leaves petiolate, apiculate, lightly tomentose | 16. <i>A. obovata</i> . |
| Leaves sessile, mucronate, densely tomentose | |
| Leaves puberulent or weakly pubescent, above or beneath. | 12. <i>A. variegata</i> . |
| Plant of open upland woods; leaves 2-5 pairs | 13. <i>A. purpurascens</i> . |
| Plant of swamp forests; leaves 6 or more pairs | |

I. *A. incarnata* L., SWAMP MILKWEED. Stem freely branching above or rarely simple, 5-15 dm tall, pilose to glabrous. Leaves numerous, opposite, lanceolate to lance-elliptic or oblanceolate, 6-15 cm long, 2-7 cm wide, pilose-hispid, especially beneath, petiolate. Umbels 4-20 or more, 2.5-4.5 cm broad, paniculate. Corolla deep rose, the lobes reflexed, 4.5-5.5 mm long; corona 2-4 mm in diam., hoods 2-4 mm long, horn longer than hood and incurved over gynostegium. Follicles smooth, 6-10 cm long, 0.8-1.2 cm broad, erect on erect pedicels. July-Sept.; Aug.-Oct. Marshes, stream banks and moist meadows; chiefly mts. and pied., less fr. on cp.

1a. ssp. *incarnata*. Plant weakly pubescent to glabrous; stem usually repeatedly branched. Leaves usually narrowly oblong- to linear-lanceolate, gradually acuminate, bluntly obtuse to truncate, tending to long-petiolate. [Va., Fla., Tenn., Ky., W. Va.] Occasional N. plants may be this subspecies.

GLOSSARY OF TERMS RELATING TO KEY

ALTERNATE	- borne singly and not opposite - in leaves, one at a node.
ANTHER	- the pollen-bearing part of the stamen.
AURICULATE	- ear-shaped.
AXILLARY	- in an axil (angle between stem and leaf).
CALYX	- the outer series of the perianth, used especially when it differs in size , shape or color from the inner or petals.
CARPEL	- a simple pistil formed from 1 sporophyll, or that part of a compound pistil formed from 1 sporophyll.
COROLLA	- the inner series of the floral envelope; collective name for petals.
CORONA	- a crown-like extension of basal portions of perianth segments (may be a modification of the anthers).
DEFLEXED	- bent or curved abruptly downward, or backward.
ELLIPTIC	- shaped like an ellipse: widest at the center and the 2 ends equal.
EXsertED	- projecting out of, beyond.
FILAMENT	- any thread-like body; used especially for that part of the stamen that supports the anther.
FOLLICLE	- a dry fruit with 1 carpel and splitting down 1 side only.
GLABROUS	- without trichomes or hairs.
GYNOSTEGIUM	- fused column of anthers and stigma.
HISPID	- provided with stiff or bristly hairs.
HOOD	- composite part of corona.
HORN	- needle-like (horn-shaped) protrusion from hood.
INFLORESCENCE-	the flowering part of a plant, almost always used for a flower cluster.
LANCEOLATE	- lance-shaped; several times longer than wide, broadest towards the base and tapering to the apex.
LAX	- loose, often used for a soft open inflorescence or for soft drooping stems or foliage.
LINEAR	- narrow and flat with sides parallel, like a blade of grass.

GLOSSARY OF TERMS RELATING TO KEY continued

- MURICATE - a surface roughened by broad or fleshy pointed spines.
- NODE - the place on a stem where leaves or branches normally originate; the place on an axis that bears other structures; any swollen or knob-like structure.
- OPPOSITE - leaves 2 at a node and situated across the stem from each other.
- OVATE - egg-shaped in outline, attached at the wide end.
- PAPILLA - a minute nipple-shaped projection.
- PAPILLOSE - bearing papillae.
- PEDICEL - the support of a single flower in a cluster, any stalk or stem between sepals and main axis.
- PEDUNCULATE - having stalk or flower cluster, or of a solitary flower when that flower is the only member of an inflorescence.
- PENDENT - hanging or declined.
- PERIANTH - corolla and calyx (not including stigma and stamens).
- PETIOLE - leaf stalk.
- PILOSE - covered with long soft trichomes.
- PISTIL - the seed producing organ, consisting usually of ovary, style and stigma.
- PUBESCENT - covered with short hairs or trichomes.
- SESSILE - without petiole or pedicel.
- SPOROPHYLL - a spore bearing leaf, often highly modified.
- STAMEN - one of the pollen bearing organs of a flower, made up of filament and anther.
- STIGMA - the part of the pistil that receives the pollen, usually at or near the pistil and mostly hairy, papillose or sticky.
- SUBOPPOSITE - nearly opposite.
- SUBSESSILE - almost sessile.
- TOMENTOSE - densely woolly or pubescent.
- TRICHOME - hair-like structure or bristle which may be simple, branched, capitate, and unicellular or multicellular.

GLOSSARY OF TERMS RELATING TO KEY continued

- UMBEL - an inflorescence with pedicels or peduncles (rays) or both arising from a common point.
- WHORLED - with 3 or more leaves or other structures arranged in a circle around a stem or some common axis.

THE MILKWEED FAMILY - ASCLEPIADACEAE

A thick white sap oozing from any cut or broken surface on plants in all except one species of milkweed (*Asclepias tuberosa*). Flower structure extraordinary, the tips of the five stamens being more or less joined with each other and also with the broad stigma, which is supported by two styles, one for each of the two ovaries. The pollen of each stamen in two waxy masses, each one connected with the mass of the adjacent stamen (further details given under *Asclepias*). The fruit a pod (follicle), two obtainable from each flower but not formed by many of the flowers in a cluster. Seeds tipped with long silky hairs.

THE MILKWEEDS (ASCLEPIAS)

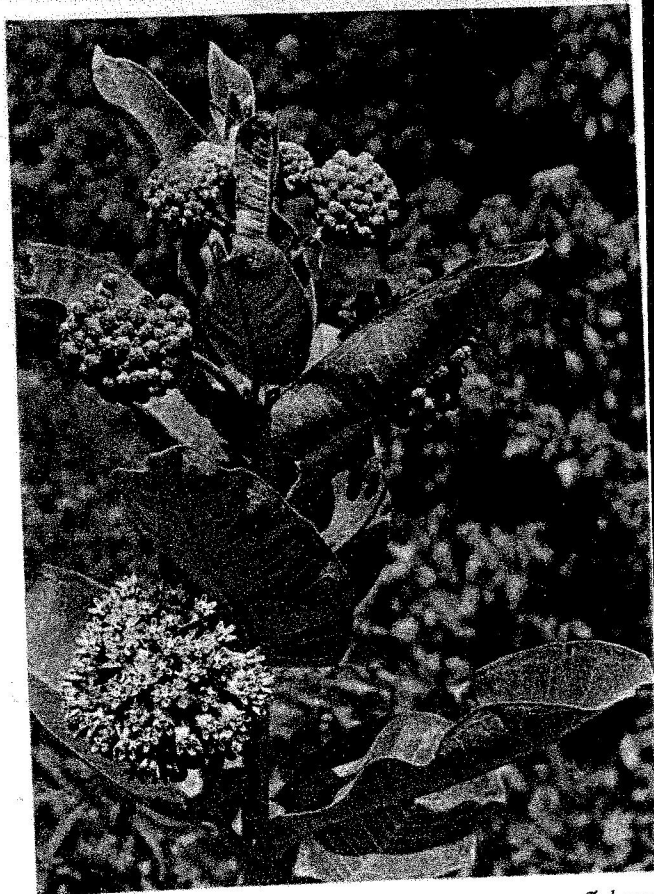
Flowers in *Asclepias* have a deeply cleft corolla, the five lobes of which are turned back and downward so as to conceal the calyx. A crown or *corona* is formed of five cups just above the junction of the corolla-lobes, each on a short tube. From within each cup rises a curved horn, its point directed toward the stigma.

The adjoining masses of pollen are tightly attached to a triangular gland that comes between them. Insects alighting on the stigma may get their feet in this. If they are large enough they can pull it out and fly away with the two masses of pollen - the grains of which can be rubbed off on the next stigma visited. The flowers are borne

in sizable clusters on stalks that all radiate from a central point, at the tip of a branch or stem, or in the axil of a leaf.

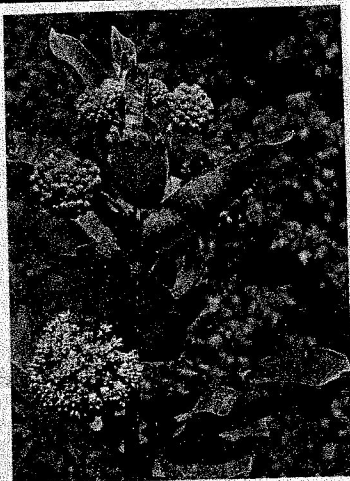
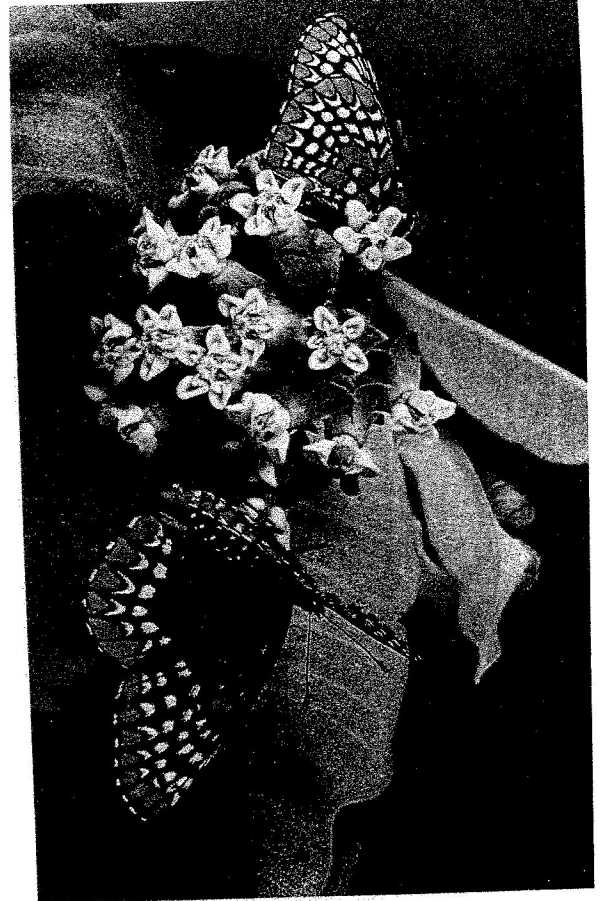
COMMON MILKWEED, *A. SYRIACA*, is seen everywhere along roadsides and in fields. Its flowers of dull crimson-purple, sometimes greenish, come in several clusters from the axils of the leaves. The fruits, which are thick, warty follicles, stand erect on stalks which are bent downward. The leaves are large (4-10 inches long), oblong, broad, and blunt, and on the under surface they are coated with a grayish down. They are borne in pairs and have very short stalks. While the stem most frequently ranges from 1 to 4 feet tall, it sometimes reaches 7 feet. June to August.

10. *A. syriaca* L.,* COMMON MILKWEED. Stem simple, usually solitary, 0.5-2 m tall, tomentulose to glabrate. Leaves opposite, widely elliptic to ovate-elliptic, 10-25 or rarely 6-30 cm long, 3-10 cm wide, thick, tomentose beneath, glabrate above. Umbels 2-6 from upper nodes, 5-10 cm broad; peduncle 3.5-8.5 cm long, pedicels 3-4 cm long. Corolla rose or greenish white, the lobes reflexed, 7-9 mm long; corona 3-6 mm in diam., lateral hood margins with a single median tooth, horns more or less equaling the hoods and arching close above the gynostegium. Follicles erect, 8-12 cm long, 2.5-3.5 cm broad, muricate. (n=11) June-Aug.; July-Sept. Meadows, fields and roadsides; primarily mts. and pied., infreq. in cp. of N.C. [Va., Ga., Tenn., Ky., W.Va.] Reported to hybridize occasionally with *A. amplexicaulis*; the hybrid is *A. intermedia* Vail.



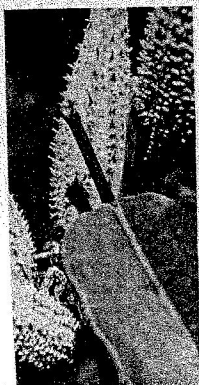
Asclepias syriaca

Johnson



Asclepias syriaca

Johnson

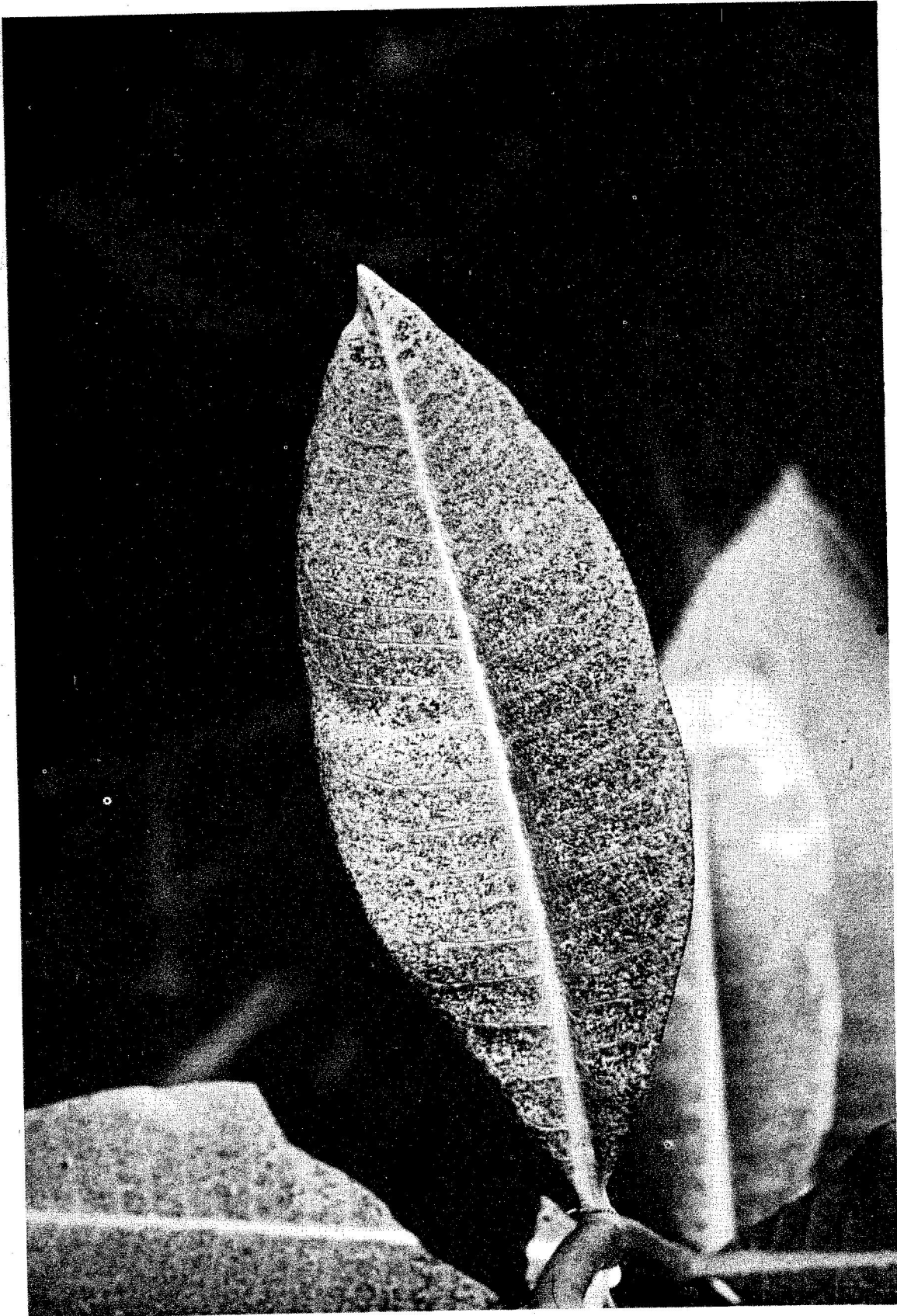
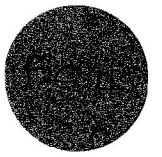


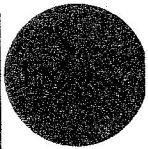
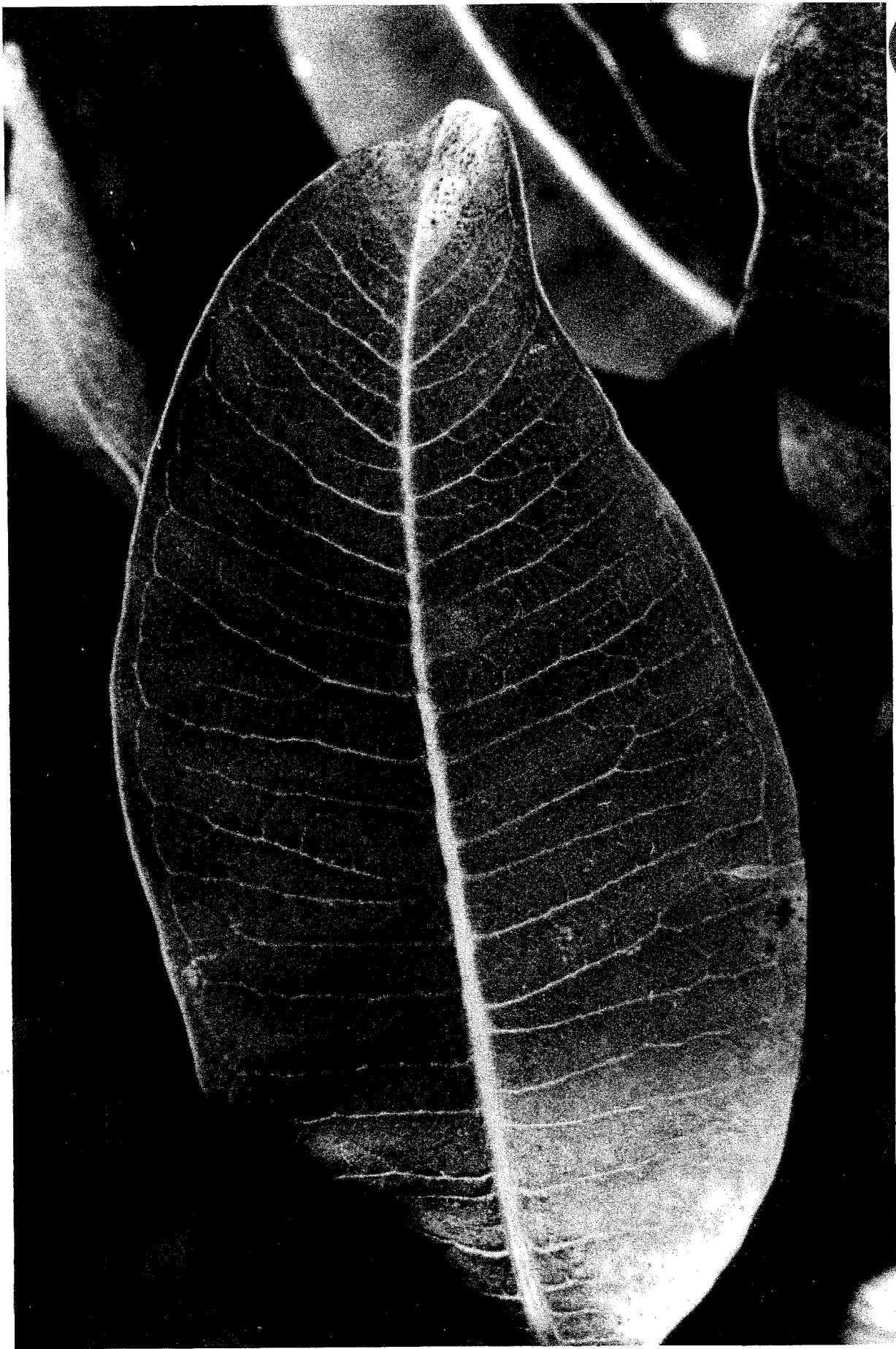
Common Milkweed
Asclepias syriaca

Fig. 1 (top left)
Whole plant

Fig. 2 (bottom left)
Seed pods and whole plant

Fig. 3 (top right)
Detail of flowering parts





EXPLANATION OF DATA SHEET

1. PLANT NUMBER: - From 1-15 (minimum of 10).
2. HEIGHT (CM): - Height of milkweed plant in centimeters, from base to apex.
3. NUMBER
LEAVES
INJURED: - Number of leaves on the plant showing ozone injury.
4. TOTAL NUMBER
LEAVES: - Total number of leaves on plant (not to exceed 20).
5. PERCENT
INJURED
LEAVES/
PLANT: - Percentage of total number of leaves showing ozone injury per plant.
6. PERCENT
LEAF AREA
INJURED: - Estimate of percentage of the total area of each individual leaf showing ozone injury.
7. MEAN %
LEAF AREA
INJURED: - Arithmetic mean of the percentage injuries.
8. POLLN. SYMP.
(SYMB): - Pollution symptom found on plant
e.g., Black/purple stippling B/P
Chlorotic stippling C
Tan flecking T
Necrotic stippling N
ENTER APPROPRIATE SYMBOL(S) ON DATA SHEET.
9. OTHER
(SYMB): - Note presence of any other injury if an appreciable quantity
e.g., wind injury W
scorching S
insect injury I
fungal injury F
or any others (denote using a symbol and explain on data sheet).
10. To summarize each plot calculate the mean and standard deviation for:
 1. The heights of plants in the plot.
 2. The percent of injured leaves per plant.
 3. The mean percent leaf area injured by ozone.

AIR QUALITY DIVISION

OZONE INJURY TO COMMON MILKWEED 1984

[illegible]

NATIONAL PARK SERVICE

AIR QUALITY DIVISION

OZONE INJURY TO COMMON MILKWEED 1984

<u>COLLECTED BY:</u> <u>DATE:</u>			<u>PLOT LOCATION:</u> NPS UNIT: _____ LOCALITY: _____ TOPO MAP: _____ PLOT NO (1-10): _____			<u>SITE CONDITIONS:</u> MOISTURE: _____ SLOPE: _____ ASPECT: _____ ELEV (FT): _____						
PLANT NUMBER	HEIGHT (CM)	NUMBER LEAVES INJURED	TOTAL NUMBER LEAVES	PERCENT OF INJURED LEAVES/PLANT	PERCENT LEAF AREA INJURED BY OZONE					MEAN % LEAF AREA INJURED	POLLN. SYMPT. (SYMB)	OTHER (SYMB)
12												
13												
14												
15												

SUMMARY OF DATA

AVERAGE PLOT SCORE	HEIGHT (CM)	PERCENT INJURED LEAVES PER PLANT	MEAN PERCENT LEAF AREA INJURY
MEAN ($\bar{x}$)			
STANDARD DEVIATION (S.D)			