Journal of
Plant Science & Research

Open Science Publications

Volume 8, Issue 1 - 2021

www.opensciencepublications.com

Ozone-Sensitivity of Seven Milkweed Species (*Asclepias* spp)

Research Article

Mercado ME[1], Decoteau DR[2], Marini RP[2] and Davis DD[3]*

[1]*Department of Plant Pathology and Environmental Microbiology, The Pennsylvania State University, USA*

[2]*Department of Plant Science, The Pennsylvania State University, USA*

[3]*Department of Environmental Microbiology, The Pennsylvania State University, USA*

***Corresponding author:** Davis DD, Department of Environmental Microbiology, The Pennsylvania State University, University Park, PA, USA, Email: ddd2@psu.edu

Article Information: Submission: 11/11/2020; **Accepted:** 11/03/2021; **Published:** 15/03/2021

Abstract

Tropospheric ozone (O_3) is considered to be the most important phytotoxic air pollutant across many parts of the USA and continues to be of major concern in natural ecosystems worldwide. Milkweed species (*Asclepias* spp) are among the most O_3-sensitive plants that occur in natural ecosystems, as well as a weed in agricultural systems. Seven milkweed species were exposed to O_3 during 2020 within controlled-environment chambers in a greenhouse. Arizona, bloodflower (syn. ornamental), and common milkweed developed the greatest O_3-induced leaf injury ("stipple") and were considered to be very sensitive to O_3.The response of purple milkweed was variable. Slimleaf milkweed exhibited low levels of leaf stipple and was considered tolerant. Horsetail and pineneedle Milkweed were considered resistant to O_3. Results were entered into a master table, which now lists the relative O_3-sensitivity of 27 of 76 (36%) milkweed species common in the USA.

Keywords: Air pollution; Ozone; CSTR exposure chambers; Milkweeds; Asclepias

Introduction

Tropospheric ozone (O_3) is considered to be the most important phytotoxic air pollutant in many parts of the United States (USA) and continues to be of major concern in natural ecosystems worldwide [1,2]. O_3 is a secondary air pollutant formed when oxides of nitrogen (NOx) and volatile organic compounds (VOCs) react in the presence of sunlight. Tropospheric concentrations vary seasonally, generally being greater in summer and minimal in the winter across the USA [1,3]. O_3 levels also vary daily, typically following diurnal patterns with concentrations low in early morning, increasing in late afternoon, and minimal at night [1,3-5].

On 1 October 2015, the US Environmental Protection Agency (EPA) strengthened the USA National Ambient Air Quality Standard (NAAQS) for O_3, reducing the level of the standard from 75 to 70 ppb [6]. This more stringent NAAQS is based on the 4th highest daily maximum of an 8-hr average O_3 concentration across 3 consecutive years. The reduction makes the new O_3 NAAQS more stringent to help protect public health and welfare in the USA, including the health of O_3-sensitive plants such as milkweed.

Milkweeds *(Asclepias* spp*)* are classified within the family Asclepiadaceae. Agrawal [7] estimated that milkweed species in the Americas numbered ~130. Woodson [8,9] listed ~105 indigenous milkweed species in North America alone. The US Department of Agriculture (USDA) now lists 76 milkweed species found growing in the USA [10]. Scientific and common names of those 76 species are used in this paper (Table 1).

Milkweed species reported to be sensitive to O_3 include common [11], bloodflower (syn. tropical) [12], poke (syn. tall) [13,14], and swamp milkweed [15]. However, most milkweed species in the USA have not been evaluated for O_3-sensitivity. Prior to this paper, only 22

Table 1: Scientific and common names of milkweed species (*Asclepias* spp) [10] used in this study. Column headings indicate method used to estimate relative O_3-sensitivity, relevant reference regarding O_3-sensitivity, common O_3-induced symptoms, and an overall subjective O_3-sensitivity rating. Blank cells indicate no data. Overall table updated and adapted from [16].

[v]Scientific name	[v]Common name	[w]Sensitivity evaluation method	[x]Selected reference	[y]Symptom response	[z]Sensitivity rating
A. albicans S. Watson	Whitestem Milkweed				
A. amplexicaulis Sm.	Clasping Milkweed				
A. angustifolia Schweigg.	Arizona Milkweed	CSTR	This Paper	STIPPLE	VS
A. arenaria Torr.	Sand Milkweed	CSTR	[15]	STIPPLE	T
A. asperula (Decne.) Woodson	Spider Milkweed	CSTR	[19]	STIPPLE	T
A. brachystephana Engelm.	Bract Milkweed				
A. californica Greene	California Milkweed	FIELD	Temple 1999	NONE	R
A. cinerea Walter	Carolina Milkweed				
A. connivens Baldw.	Largeflower Milkweed				
A. cordifolia (Benth.) Jeps.	Heartleaf Milkweed	CSTR	[15]	NONE	R
A. cryptoceras S. Watson	Pallid Milkweeed	FIELD	Davis 2017	NONE	R
A. curassavica L.	Bloodflower syn. Tropical Milkweed	CSTR	Hughes et al. 1990	STIPPLE	VS
A. curtissii A. Gray	Curtiss' Milkweed				
A. cutleri Woodson	Cutler's Milkweed				
A. emoryi (Greene) Vail	Emory's Milkweed				
A. engelmanniana Woodson	Engelmann's Milkweed	CSTR	Davis, Unpub.	STIPPLE	T
A. eriocarpa Benth.	Woolypod Milkweed	CSTR	[15]	STIPPLE	S
A. erosa Torr.	Desert Milkweed				
A. exaltata L.	Poke syn. Tall Milkweed	FIELD, OTC	Neufeld et al. 1992	STIPPLE	VS
A. fascicularis Decne.	Mexican Whorled Milkweed	CSTR	[15]	NONE	R
A. feayi Chapm.	Florida Milkweed				
A. fructicosa L.	African Milkweed				
A. glaucescens Kunth	Nodding Milkweed				
A. hallii A. Gray	Hall's Milkweed				
A. hirtella (Pennell) Woodson	Green Milkweed	CSTR	[19]	NONE	R
A. humistrata Walter	Pinewoods Milkweed				
A. hypoleuca (A. Gray) Woodson	Mahogany Milkweed				
A. incarnata L.	Swamp Milkweed	CSTR	[14]	STIPPLE	VS
A. involucrata Engelm.	Dwarf Milkweed				
A. labriformis M.E. Jones	Utah Milkweed	CSTR	[15]	NONE	R
A. lanceolata Walter	Fewflower Milkweed				
A. lanuginosa Nutt.	Sidecluster Milkweed				
A. latifolia (Torr.) Raf.	Broadleaf Milkweed	CSTR	[15]	NONE	R
A. lemmonii A. Gray	Lemmon's Milkweed				
A. linaria Cav.	Pineneedle Milkweed	CSTR	This Paper	STIPPLE	T
A. linearis Scheele	Slim Milkweed				
A. longifolia Michx.	Longleaf Milkweed				
A. macrotis Torr.	Longhood Milkweed				
A. meadii Torr.	Mead's Milkweed				
A. michauxii Decne.	Michaux's Milkweed				
A. nivea L.	Caribbean Milkweed				
A. nummularia Torr.	Tufted Milkweed				
A. nyctaginifolia A. Gray	Mojave Milkweed				
A. obovata Elliott	Pineland Milkweed				
A. oenotheroides Schltdl. & Cham.	Zizotes Milkweed				
A. ovalifolia Decne.	Oval-leaf Milkweed	CSTR	[19]	STIPPLE	T
A. pedicellata Walter	Savannah Milkweed				
A. perennis Walter	Aquatic Milkweed				
A. physiocarpa (E. Mey.) Schitr.	Balloonplant				
A. prostrata W.H. Blackw.	Prostrate Milkweed				
A. pumila (A. Gray) Vail	Plains Milkweed				

Citation: Mercado ME, Decoteau DR, Marini RP, Davis DD. Ozone-Sensitivity of Seven Milkweed Species (*Asclepias* spp). J Plant Sci Res. 2021;8(1): 201

A. purpurascens L.	Purple Milkweed	CSTR	This Paper	STIPPLE	T/R
A. quadrifolia Jacq.	Fourleaf Milkweed				
A. quinquedentata A. Gray	Slimpod Milkweed				
A. *rubra* L.	Red Milkweed				
A. rusbyi (Vail) Woodson	Rusby's Milkweed				
A. scaposa Vail	Bear Mountain Milkweed				
A. solanoana Woodson	Serpentine Milkweed				
A. speciosa Torr.	Showy Milkweed	CSTR	[19]	STIPPLE	VS
A. sperryi Woodson	Sperry's Milkweed				
A. stenophylla A. Gray	Slimleaf Milkweed	CSTR	This Paper	STIPPLE	T/R
A. subulata Decne.	Rush Milkweed				
A. subverticillata (A. Gray) Vail	Horsetail Milkweed	CSTR	This Paper	NONE	R
A. sullivantii Engelm.	Prairie Milkweed	CSTR	[19]	STIPPLE	T
A. syriaca L.	Common Milkweed	OTC	[10]	STIPPLE	VS
A. texana A. Heller	Texas Milkweed				
A. tomentosa Elliott	Tuba Milkweed				
A. tuberosa L.	Butterfly Milkweed	CSTR	[19]	NONE	R
A. uncialis Greene	Wheel Milkweed				
A. variegata L.	Redring Milkweed				
A. verticillata L.	Whorled Milkweed	CSTR	[19]	NONE	R
A. vestita Hook. &Arn.	Wooly Milkweed				
A. viridiflora Raf.	Green Comet Milkweed	CSTR	[15]	STIPPLE	T
A. *viridis* Walter	Green Antelopehorn Milkweed	CSTR	[15]	STIPPLE	T
A. viridula Chapm.	Southern Milkweed				
A. welshii N.H. Holmgren & P.K. Holmgren	Welsh's Milkweed				

[v]Scientific and common names from USDA PLANTS Database Profiles
[w]Methodology used to rate sensitivity to ozone: CSTR = Continuous Stirred Tank Reactor, FIELD = field surveys, OTC = Open-top chambers
[x]Reference = one selected reference
[y]Symptom: STIPPLE = adaxial leaf surface stipple, NONE = no symptom
[z]Sensitivity rating: VS = Very Sensitive, suitable as bioindicator; S = Sensitive, readily visible stipple; T=Tolerant, trace of extremely light stipple; R =Resistant,no stipple; Blank cell = no information

of the 76 (29%) milkweed species listed in Table 1 had been evaluated for O_3-sensitivity [16].

The objective of this study was to evaluate the O_3-sensitivity of 7 milkweed species and to determine or confirm their relative sensitivity or resistance by updating a master list previously compiled by the authors [16,17].

Materials and Methods

Ozone exposure chambers

O_3 exposures were conducted within 16 continuous stirred tank reactor (CSTR) chambers [18] in a greenhouse on the University Park campus of The Pennsylvania State University (40°48'20"N, 77°51'08"W).O_3 concentrations within each chamber were monitored using a photometric O_3 analyzer (Model 49,Thermo Environmental Corp., Franklin, MA). O_3 treatments were administered, monitored, and controlled via a computerized system during exposures, which also monitored and displayed temperature and relative humidity in each chamber [19].

Exposure study

Plant Culture: Plant culture followed protocols of Myers et al. [20]. Arizona, bloodflower, common, horsetail, pineneedle, purple, and slimleaf milkweed were selected based on availability of seeds. Common milkweed was included as a standard, since this species is known to be highly sensitive to O_3 [11,12]. During August 2020, milkweed seeds were placed in 2-L pots containing moist potting mix with ~1g slow-release fertilizer (15N-4P-10K) and watered as needed. Resultant seedlings were grown in a greenhouse containing air that passed through activated-charcoal filters, reducing the ambient greenhouse O_3 to ~5 ppb.

Plant exposure to O_3: O_3 exposures began in mid-September 2020. Two O_3 treatments were used (elevated vs control). Our target value for the elevated O_3 concentration was 75 ppb since various milkweed species had exhibited classic O_3-induced leaf stipple following exposure to this concentration during previous studies conducted within these chambers [17,20]. However, the mean O_3 concentration within the exposure chambers was better controlled at ~77 ppb, which was thus used in this study. The average O_3 concentrations in the control chambers were ~5 ppb, which was the lowest concentration that could be attained using the charcoal-filter system that scrubbed the air within both the greenhouse and control chambers. Fourteen chambers were maintained at ~77 ppb O_3 and 2 control chambers were kept at~5 ppb O_3. We had planned to put 2 plants/species in each chamber. However, due to reduced seed germination and decreased seedling survival, the number of plants/species varied from 0-2/chamber.

Citation: Mercado ME, Decoteau DR, Marini RP, Davis DD. Ozone-Sensitivity of Seven Milkweed Species (*Asclepias* spp). J Plant Sci Res. 2021;8(1): 201

Milkweed plants were exposed for 7 weeks, 6 days/week, 8h/day (0900-1700 hours), in a square-wave design. During exposures, mean air temperature was ~25ºC and relative humidity was ~44%. Once a week, plants were watered, redistributed randomly within each chamber, and O_3-induced foliar stipple evaluated. Following each weekly 6-day exposure, plants remained within the same CSTR exposure chambers with the chamber doors open until the next exposure began.

Leaf injury (stipple) evaluation: O_3-induced foliar stipple on the upper leaf surface/plant was visually estimated [17,20] within each chamber using a modified Horsfall-Barratt scale [21]. Symptom rating classes in this scale ranged from 0 to 5, where 0 = no injury, 1 = 1-6% injury, 2 = 7-25%, 3 = 26-50%, 4 = 51-75%, and 5 = 76-100% injury. To provide a single value for statistical analyses, the midpoint of each class was calculated as the mean of the minimum and maximum percentage.

Data Analyses: Analyses of O_3 response data were conducted using a repeated measures analysis in a randomized complete block design as described in SAS PROC GLIMMIX [22]. Chamber was the block; random effects included chamber and plants nested in chamber. Since the (week x species) interaction was significant (P = 0.0001), the Least Squares means (LS means) for species within week were statistically compared using the SLICE DIFF Option at P = 0.05. LS means can be defined as a linear combination (sum) of the estimated effects (means) from the linear model. When the data contain no missing values, the LS means and arithmetic means are identical. When missing values occur, the two will differ and the LS means are preferred, because they reflect the model that was used to fit the data. When the data are extremely unbalanced, as in this study, the estimated LS means may differ greatly from the arithmetic means, because there are too few observations to make appropriate adjustments. Since the standard errors from the repeated measures are correct, the multiple comparisons for LS means in Table 2 are correct, and the arithmetic means are presented in Figure 1.

Results

Results based on Least Squares means: Control plants did not exhibit O_3-induced foliar injury (data not shown). Foliar injury was not observed on any species following 1 week of exposure to ~77 ppb O_3. At 2 weeks, light stipple was noted on Arizona milkweed and extremely light stipple on horsetail milkweed (Table 2). Following 3 weeks of exposure, more severe O_3 injury was observed on Arizona, bloodflower, common, purple, and slimleaf milkweed. At 3-4 weeks, stipple was noted on all species except horsetail and pineneedle milkweed. Following 5-7 weeks exposure, stipple occurred on all species except horsetail milkweed. Slimleaf milkweeds in both the control and exposure chambers began to die from unknown causes at 5 weeks and all were dead by 7 weeks.

Table 2: Least square means (LS means) of O_3 injury on 7 milkweed species exposed to ~77 ppb O_3 for 1-7 weeks. Injury not observed on controls (data not shown). LS means based on a repeated measures analysis in a randomized complete block design via SAS PROC GLIMMIX. LS means for species within week are compared using the SLICE DIFF Option at *P* = 0.05.

	Weeks of exposure						
Species	1	2	3	4	5	6	7
Arizona	0	4.8	12.0a[z]	22.5a	25.2a	28.7a	36.9a
Common	0	0	6.5a	12.4b	13.0b	17.0b	29.8b
Bloodflower	0	0	0.7b	1.4c	4.2b	12.7b	27.5b
Purple	0	0	0.6b	2.7c	10.5b	11.9b	25.2b
Slimleaf	0	0	4.1b	9.5b	8.2b	6.8bc	- - -
Pineneedle	0	0	0.0b	0.0c	0.1c	0.1c	0.1c
Horsetail	0	2.2	0.0b	0.0c	0.0c	0.0c	0.0c

[z] Values within columns followed by common letters do not differ at *P* = 0.05

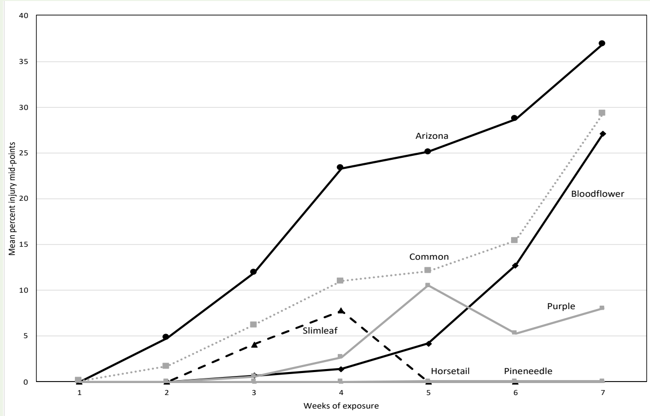

Figure 1: Relationship between mean % O_3 injury, based on original injury mid-points, on leaves of 7 milkweed species exposed to ~77 ppb O_3 for 1-7 weeks. Injury was not observed on controls (data not shown). Slimleaf milkweed plants were dead from unknown cause(s) in week 7 and not rated. Response lines of horsetail, pineneedle, and slimleaf milkweed for weeks 5 and 6 were indistinguishable from the horizontal X-axis line.

Although PROC GLIMMIX estimated the LS means for slimleaf milkweed on week 7, the value is questionable since there were no living slimleaf milkweed plants at this time (Table 2). After the 7th and final rating, O_3 injury based on LS mean mid-point ratings of the various milkweed species, from greatest to least, was: [Arizona (36.9%)>common (29.8%)=bloodflower (27.5%)=purple (25.2%)>pineneedle (0.1%)=horsetail (0.0%), and slimleaf (undetermined due to 100% mortality)](Table 2).

Results based on mid-point arithmetic means: Injury ratings for Arizona milkweed were greater than all other species as early as week 2, and increased nearly linearly through the study (Figure 1). The injury ratings for common and bloodflower milkweeds increased slowly for the first 5 weeks before increasing more rapidly in the final 2 weeks.

Discussion

Seven milkweed species were exposed to O_3 during 2020. As shown in Table 1, Arizona, bloodflower, and common milkweeds developed the greatest O_3-induced leaf injury ("stipple") and were classified as "Very Sensitive". The response of purple milkweed was variable between Table 2 and Figure 1 and was classified as "Tolerant", but will be exposed again in the next study using more plants. Slimleaf milkweed exhibited very low levels of leaf stipple and was considered "Tolerant". Horsetail and pineneedle milkweed exhibited either a trace or no symptoms and were classified as "Resistant". Slimleaf milkweeds in both the control and exposure chambers began to die from unknown cause(s) at 5 weeks (Figure 1). However, there was

Citation: Mercado ME, Decoteau DR, Marini RP, Davis DD. Ozone-Sensitivity of Seven Milkweed Species (*Asclepias* spp). J Plant Sci Res. 2021;8(1): 201

a trace of accumulated stipple on living slimleaf milkweeds after 4 weeks of exposure, so the species was classified as slightly “Sensitive” to O_3 (Table 1).

Milkweed species confirmed to be sensitive to O_3 based on previous reports include common [11] and bloodflower [12]. Common milkweed is a widely used bioindicator of phytotoxic levels of tropospheric O_3 across the USA [11, 17]. Bloodflower milkweed is likewise very sensitive to O_3, but may not be acceptable to use as a bio indicator in some regions of the USA since it may contain a protozoan that is pathogenic to some butterflies, including the popular monarch butterfly [7,17].

An important finding in this study, is that we reported for the first time that Arizona milkweed is very sensitive to O_3. The species was the most sensitive of the 7 milkweed species from week 2 to week 7 (Figure 1). Arizona milkweed is rare in the USA, being found only in southern Arizona (and into northern Mexico), where it grows in arroyos, canyons, along streambeds, and on slopes (http://southwestdesertflora.com/WebsiteFolders/All_Species/Asclepiadaceae/Asclepias%20angustifolia,%20Arizona%20).

The high O_3-sensitivity of Arizona milkweed to O_3 is significant, since it was injured by ~77 ppb O_3, a concentration that occurs in the USA troposphere. In fact, in 2020 the US EPA reported measuring ambient O_3 concentrations as high as 90 ppb in Denver, CO, likely a result of O_3-forming precursors in the smoke of the 2020 widespread wildfires in southwestern USA (https://www.denverpost.com/2020/08/25/colorado-wildfire-smoke-pollution-ozone/, accessed 10 January 2021). The high sensitivity of Arizona milkweed was greater than that of common milkweed, a frequently used bio indicator to detect phytotoxic levels of ambient ozone [11,17]. Arizona milkweed may be an excellent, new bio indicator to detect phytotoxic levels of O_3 in the arid Southwest, if its high sensitivity to O_3 is maintained in dry regions. In dry areas, droughts can induce stomatal closure, which in turn reduce gas uptake, including uptake of O_3 and subsequent foliar injury [22], confounding O_3 injury surveys.

Findings from this study added 5 new milkweed species to Table 1 and confirmed the ratings of 2 others. Milkweed species, such as Arizona milkweed, which are classified as “Very Sensitive” to O_3, should be considered for possible use as bio indicators to detect phytotoxic concentrations of ambient O_3. Table1 now lists the relative O_3-sensitivity of 27 of 76 (36%) milkweed species. We present this updated list in hopes that other air pollution researchers will expand and improve the ratings by evaluating the O_3 sensitivity of additional milkweed species during field surveys used in combination with controlled O_3 exposures [17].

Acknowledgements

We acknowledge funding from the Pennsylvania Department of Environmental Protection, Bureau of Air Quality, Harrisburg, PA, USA and The Pennsylvania State University, University Park, PA, USA. This work was also supported by the USDA National Institute of Food and Agriculture (NIFA) and Federal Appropriations under Project PEN04564, Accession number 1002837.

References

1. Krupa SV (1997) Air Pollution, People and Plants. Amer Phytopath Soc, St. Paul, MN 197.
2. Skelly JM (2000) Tropospheric ozone and its importance to forest and natural plant communities of the northeastern United States. Northeast Natural 7: 221-236.
3. Krupa SV, McGrath MT, Andersen CP, Booker FL, Burkey KO, et al. (2001) Ambient ozone and plant health. Plant Dis 85: 4-12.
4. United States Environmental Protection Agency (USEPA) (2003) Ozone: good up high bad nearby. Wash.
5. United States Environmental Protection Agency (USEPA) (2013) Final Report: Integrated Science Assessment of Ozone and Related Photochemical Oxidants. Report No. EPA/600/R-10/076F, Wash 1251
5. United States Environmental Protection Agency (USEPA) (2015) National Ambient Air Quality Standards (NAAQS) for Ozone.
6. Forister ML (2017) Monarchs and Milkweed: A Migrating Butterfly, a Poisonous Plant, and Their Remarkable Story of Coevolution. By Anurag Agrawal. Princeton (New Jersey): Princeton University Press.
7. Woodson RE (1941) The North American Asclepiadaceae I. Perspective of the Genera. Annals Missouri Bot Gard 28:193-244.
8. Woodson RE (1954) The North American Species of *Asclepias* L. Annals Missouri Bot Gard 41: 1-211.
9. United States Department of Agriculture (USDA) (2017) Natural Resources Conservation Service, Plants Database, Name Search: *Asclepias*, Subordinate Taxa.
10. Duchelle SF, Skelly JM (1981) Response of common milkweed to oxidant air pollution in the Shenandoah National Park in Virginia. Plant Dis. 65: 661-663.
11. Bolsinger M, Lier ME, Lanskey DM, Hughes PR(1991) Plant-herbivore interactions. Part 1: Biochemical changes in ornamental milkweed (*Asclepias curassavica* L. Asclepiadaceae) induced by ozone. Environ Pollut 72: 69-83.
12. Chappelka AH, Somers GL, Renfro JR (2007) Temporal patterns of foliar ozone symptoms on tall milkweed (*Asclepias exaltata* L) in Great Smoky Mountains National Park. Environ Pollut 149: 358-365.
13. Chappelka AH, Renfro JR, Somers GL, Nash B (1997) Evaluation of ozone injury on foliage of black cherry (*Prunus serotina*) and tall milkweed (*Asclepias exaltata*) in Great Smoky Mountains National Park. Environ Pollut 95:13-18.
14. Orendovici T, Skelly JM, Ferdinand JA, Savage JE, Sanz M-J, et al. (2003) Response of native plants of northeastern United States and southern Spain to ozone exposures;determining exposure/response relationships. Environ Pollut 125: 31-40.
15. Mercado M, Decoteau DR, Marini RP, Davis DD (2020) Ozone-sensitivity of ten milkweed species (*Asclepias* spp). Northeast Natural 27: 656-668.
16. Davis DD, Decoteau DR (2018) A review: effect of ozone on milkweeds (*Asclepias* spp.) in USA and potential implications for monarch butterflies. J Agric Env Sci 7: 76-89.
17. Heck WW, Philbeck RB, Dunning JA (1978) A Continuous Stirred Tank Reactor (CSTR) System for exposing plants to gaseous contaminants: principles, specifications, construction and operation. USDA-ARS Pub. No. ARS-S-181, New Orleans, LA.
18. Lloyd KL, Davis DD, Marini RP, Decoteau DR (2018) Effects of nightime ozone treatment at ambient concentrations on sensitive and resistant snap bean genotypes. J Amer Soc Hort Sci 143: 22-33.
19. Myers AC, Decoteau DR, Marini RP, Davis DD (2018) Sensitivity of eleven milkweed (*Asclepias*) species to ozone. North Natural 25: 265-276.
20. Horsfall JG, Barratt RW (1945) An improved grading system for measuring plant disease. Phytopathology 35: 655.
21. Littell RC, Milliken GA, Stroup WW, Wolfinger RD, and Schabenberger O (2006) SAS for mixed models 2nd Ed SAS Institute Inc Cary NC.
22. Showman RE (1991) A comparison of ozone injury to vegetation during moist and drought years. J Air Waste Mgt Assoc 41: 63-64.

Citation: Mercado ME, Decoteau DR, Marini RP, Davis DD. Ozone-Sensitivity of Seven Milkweed Species (*Asclepias* spp). J Plant Sci Res. 2021;8(1): 201