*J. Aquat. Plant Manage.* 51: 45–48

# Use of a small-scale primary screening method to predict effects of flumioxazin and carfentrazone-ethyl on native and invasive, submersed plants

LEEANN M. GLOMSKI AND MICHAEL D. NETHERLAND*

## ABSTRACT

When evaluating potential use patterns of new aquatic herbicides, it is important to determine effects on target as well as nontarget vegetation. Small-scale primary screens that provide data on the relative sensitivity of a species to a given herbicide or herbicide use rate can be used to enhance the design of more-costly and time-consuming, large-scale, growth-chamber and mesocosm studies. Flumioxazin (2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2H-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3(2H)-dione) and carfentrazone-ethyl (ethyl α,2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoate) are protoporphyrinogen oxidase (protox)–inhibiting herbicides recently registered by the U.S. Environmental Protection Agency (USEPA) for use in aquatic sites. These protox inhibitors disrupt chlorophyll synthesis and result in formation of oxygen radicals that damage cell membranes, causing them to leak cell contents. This leakage of electrolytes was measured and used to determine herbicide injury following exposure to protox inhibitors. Electrolyte leakage was measured at 5 d posttreatment for 15 submersed aquatic plant species exposed to flumioxazin at 0, 200, and 400 µg ai $L^{-1}$ (0, 0.0000265, and 0.000053 ppb) and to carfentrazone-ethyl at 0 and 200 µg ai $L^{-1}$ in small-scale laboratory assays. In these assays, flumioxazin significantly increased electrolyte leakage for coontail (*Ceratophyllum demersum* L.) and curlyleaf pondweed (*Potamogeton crispus* L.). Eurasian watermilfoil (*Myriophyllum spicatum* L.), fanwort (*Cabomba caroliniana* Gray), hydrilla [*Hydrilla verticillata* (L. f.) Royle], longbeak buttercup (*Ranunculus longirostris* Godr.), springtape (*Sagittaria kurziana* Gluck), variable watermilfoil (*Myriophyllum heterophyllum* Michx.), and waterstargrass [*Heteranthera dubia* (Jacq.) MacM.]. Species that were not significantly affected by flumioxazin included American pondweed (*Potamogeton nodosus* Poir.), common elodea (*Elodea canadensis* Michx.), Illinois pondweed (*Potamogeton illinoensis* Morong), sago pondweed [*Stuckenia pectinatus* (L.) Boerner], southern naiad [*Najas guadalupensis* (Spreng.) Magnus], and American eelgrass (*Vallisneria americana* Michx.). Of the species tested, carfentrazone-ethyl only increased electrolyte leakage of coontail, Eurasian watermilfoil, and variable watermilfoil.

*Key words*: electrolyte leakage, herbicide screening, protoporphyrinogen oxidase inhibitor.

## INTRODUCTION

During the process of evaluating potential use patterns of new aquatic herbicides, it is important to determine concentrations that affect target as well as nontarget vegetation (Netherland et al. 2005, Getsinger et al. 2008). Five new herbicides have been registered in the aquatic market since 2005, and determination of both efficacy and nontarget plant selectivity remains critical in developing use patterns for these products. Conventional, indoor, walk-in growth-chamber, greenhouse, and outdoor-mesocosm studies require large spaces, significant time (2 to 4 mo), and labor resources, and study designs are typically limited by the number of aquariums or mesocosm tanks in the study space (Getsinger et al. 2008). Small-scale, primary screens that provide initial data on the relative sensitivity of a species to a given herbicide can enhance the design of larger-scale, growth-chamber and mesocosm studies by providing valuable initial information regarding herbicide use rates or exposure times. Given the cost, time, and limited number of replicates available in the larger systems, information that can improve study design is of significant value. Small-scale screens have been used to determine the activity of the gibberellin synthesis inhibitors, triazines, and bleaching herbicides against hydrilla [*Hydrilla verticillata* (L. f.) Royle], Eurasian watermilfoil (*Myriophyllum spicatum* L.), and submersed native species; however, methods for screening rapid-acting compounds, such as protoporphyrinogen oxidase (protox) inhibitors, against submersed aquatic plants have not been developed (Netherland and Lembi 1992, Glomski and Netherland 2011).

Flumioxazin (ethyl α,2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoate) and carfentrazone-ethyl (ethyl α,2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoate) are protox-inhibiting herbicides originally registered for broadleaf

*First author: Research Biologist, U.S. Army Engineer Research and Development Center, Lewisville Aquatic Ecosystem Research Facility, 201 E. Jones Street, Lewisville, TX 75057. Second author: Research Biologist, U.S. Army Engineer Research and Development Center, Center for Aquatic and Invasive Plants, Gainesville, FL 32653. Corresponding author's E-mail: LeeAnn.M.Glomski@usace.army.mil. Received for publication November 15, 2011 and in revised form November 16, 2012.

weed control in terrestrial systems (Senseman 2007); however, both have been approved for use in aquatic sites in recent years. Protox inhibitors disrupt chlorophyll synthesis by competing with protoporphyrinogen for binding sites on the protoporphyrinogen oxidase enzyme. Without available binding sites, protoporphyrinogen leaks into the cytoplasm and is converted to protoporphyrin IX when exposed to light. Protoporphyrin IX then reacts with oxygen to form singlet oxygen radicals that damage cell membranes causing them to leak (Hess 2000, Senseman 2007). This leakage of electrolytes has been measured and used to determine herbicide injury of membrane-disrupting and photosynthesis-inhibiting herbicides (Vanstone and Stobbe 1977, Yanase et al. 1990, Koo et al. 1994, Li et al. 2000, Falk et al. 2006, Koschnick et al. 2006), freezing resistance and frost tolerance (Dexter et al. 1932, Sukumaran and Weiser 1972, Nunes and Smith 2003), and seed vigor (Duke et al. 1983). The objective of this study was to determine whether electrolyte leakage measures could be used in small-scale rapid assays to determine the relative sensitivity of native and nonnative, submersed aquatic plants to flumioxazin and carfentrazone-ethyl.

## MATERIALS AND METHODS

To determine the effect of flumioxazin and carfentrazone-ethyl on submersed plants, small-scale assays were conducted in reach-in growth chambers at the U.S. Army Research and Development Center's Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX. Studies were conducted using a Percival E-36L growth chamber[1] set at 25 C (77 F) and a 16 : 8 light : dark photoperiod. Light intensity was 182 μmol photons $m^{-2}$ $s^{-1}$. An incubation medium of 1 mM 2-(morpholino) ethanesulfonic acid buffer (MES) and 2% (w/v) sucrose was prepared, and the pH of the medium was adjusted to 6.5 with 2.1 N NaOH (Duke and Kenyon 1993, Kenyon et al. 1985). Twenty milliliters (0.68 oz) of medium were added to 50-ml centrifuge tubes along with 0.25 g of fresh plant tissue. Because the more tissue per tube, the more sensitive the assay is (Duke and Kenyon 1993), the weight used in these assays was enough to fill the tube without overpacking it. To allow plants to acclimate to a different light regime, during fall and winter, when light intensity was less in greenhouse and outdoor conditions, plants were allowed to acclimate for 2 to 3 d in the growth chamber before being used in the assay. Treatments applied to tubes included 200 and 400 μg ai $L^{-1}$ (0.0000265 and 0.000053 ppb) flumioxazin[2] or 200 μg ai $L^{-1}$ carfentrazone-ethyl[3] and an untreated control. A linear rate response has not been seen in previous studies with protox inhibitors; therefore, only the maximum use rate of carfentrazone-ethyl was used in these assays (Glomski et al. 2006, Glomski and Netherland 2007). Treatments were replicated four times. Conductivity readings were taken on each tube after 5 d of herbicide exposure using an Accumet AP85 pH/conductivity meter.[4] Tubes were then boiled for 1 h at 90 C and then quickly cooled to 25 C before final conductivity readings were taken to determine total electroconductivity of the tissue. Tubes were shaken for 5 s before readings were taken. Percentage of electrolyte leakage was calculated using the following equation (Falk et al. 2006):

$$(Conductivity\ before\ boiling / Conductivity\ after\ boiling) \times 100$$

Species tested included: American pondweed (*Potamogeton nodosus* Poir.), coontail (*Ceratophyllum demersum* L.), curlyleaf pondweed (*Potamogeton crispus* L.), common elodea (*Elodea canadensis* Michx.), Eurasian watermilfoil, fanwort (*Cabomba caroliniana* Gray), hydrilla, Illinois pondweed (*Potamogeton illinoensis* Morong), longbeak buttercup (*Ranunculus longirostris* Godr.), sago pondweed [*Stuckenia pectinatus* (L.) Boerner], southern naiad [*Najas guadalupensis* (Spreng.) Magnus], springtape (*Sagittaria kurziana* Gluck), variable watermilfoil (*Myriophyllum heterophyllum* Michx.), waterstargrass [*Heteranthera dubia* (Jacq.) MacM.], and American eelgrass (*Vallisneria americana* Michx.). Fanwort and sago pondweed were not available when carfentrazone-ethyl assays were conducted. Assays were repeated in time, and flumioxazin data were subjected to 1-way ANOVA with means compared via the Student–Newman–Keuls method (SNK; $\alpha = 0.05$). Carfentrazone-ethyl data were subjected to *t* tests ($\alpha = 0.05$). When no interaction was detected between repeat experiments, data were pooled.

TABLE 1. MEAN (± SE) PERCENTAGE OF ELECTROLYTE LEAKAGE OF NATIVE SUBMERSED PLANTS EXPOSED TO FLUMIOXAZIN FOR 5 D. EACH SPECIES WAS EXAMINED TWICE, AND DATA WERE POOLED. SPECIES IN ITALICS ARE INVASIVE PLANTS. EACH MEAN REPRESENTS THE AVERAGE OF 8 REPLICATE TREATMENTS. MEANS SHARING THE SAME LETTER IN EACH ROW DO NOT SIGNIFICANTLY DIFFER FROM EACH OTHER. DATA WERE SUBJECTED TO A 1-WAY ANOVA, AND MEANS WERE SEPARATED USING THE STUDENT–NEWMAN–KEULS (SNK) METHOD ($\alpha = 0.05$).

| Species | Control | 200 μg $L^{-1}$ | 400 μg $L^{-1}$ |
|---|---|---|---|
| Not sensitive | | | |
| American eelgrass | 18.7 ± 2.7 | 23.8 ± 2.2 | 25.0 ± 5.3 |
| American pondweed | 47.1 ± 1.6 | 54.3 ± 9.2 | 54.4 ± 7.4 |
| Common elodea | 43.4 ± 1.8 | 47.2 ± 1.8 | 50.4 ± 2.1 |
| Illinois pondweed | 37.8 ± 4.2 | 40.9 ± 7.3 | 31.4 ± 1.3 |
| Sago pondweed | 46.0 ± 2.7 | 43.6 ± 1.9 | 39.4 ± 0.9 |
| Southern naiad | 38.6 ± 2.6 | 49.9 ± 4.5 | 43.2 ± 4.5 |
| Sensitive | | | |
| Coontail | 23.6 ± 1.5 a | 92.3 ± 2.5 b | 91.1 ± 2.5 b |
| *Curlyleaf pondweed* | 53.2 ± 3.0 a | 82.1 ± 1.1 b | 78.6 ± 1.6 b |
| *Eurasian watermilfoil* | 23.8 ± 4.0 a | 95.0 ± 0.9 b | 97.0 ± 0.3 b |
| Fanwort | 23.0 ± 1.3 a | 82.2 ± 5.4 b | 87.6 ± 2.3 b |
| *Hydrilla* | 25.9 ± 5.0 a | 83.9 ± 8.7 b | 81.6 ± 8.7 b |
| Longbeak buttercup | 55.9 ± 3.6 a | 63.9 ± 2.7 a | 76.2 ± 1.5 b |
| Springtape | 14.7 ± 1.5 a | 15.6 ± 1.9 a | 30.8 ± 5.9 b |
| Variable watermilfoil | 47.7 ± 0.9 a | 51.7 ± 0.4 a | 56.6 ± 2.6 b |
| Waterstargrass | 21.3 ± 2.2 a | 81.7 ± 2.4 b | 79.7 ± 2.1 b |

## RESULTS AND DISCUSSION

Six of the 15 species tested showed no change in electrolyte leakage when exposed to either rate of flumioxazin (Table 1). Of those that were sensitive, coontail and Eurasian watermilfoil were the most sensitive, leaking more than 90% of their electrolytes. Species leaking between 30 and 90% included curlyleaf pondweed, fanwort, hydrilla, longbeak buttercup, springtape, variable watermilfoil, and waterstargrass (Table 1). In most cases, doubling the rate of flumioxazin from 200 to 400 μg ai $L^{-1}$ did not cause an increase in electrolyte leakage. This lack of a rate response has also been reported for hydrilla treated with flumioxazin and for

variable watermilfoil and Eurasian watermilfoil treated with carfentrazone-ethyl (Glomski et al. 2006, Glomski and Netherland 2007, Mudge et al. 2010). Traditional concentration and exposure relationships may not explain the activity of these rapid-acting protox-inhibiting herbicides. Carfentrazone-ethyl significantly increased electrolyte leakage of coontail, Eurasian watermilfoil, and variable watermilfoil (Table 2). Coontail was the most sensitive, followed by Eurasian watermilfoil and variable watermilfoil with 92, 66 and 56% electrolyte leakage, respectively. The other species evaluated did not show increased electrolyte leakage following exposure to carfentrazone.

Six of the species tested in these assays have also been tested in greenhouse or outdoor mesocosm trials and 5 of the 6 species were tested under similar pH levels. Similar to results from this assay, coontail, fanwort, and hydrilla were sensitive to flumioxazin in large-scale trials, whereas American eelgrass was not (Bultemeier et al. 2009, Mudge et al. 2010). Large-scale trials also found that Eurasian watermilfoil, fanwort, and variable watermilfoil were susceptible to carfentrazone-ethyl, which supports the outcome of these assays (Glomski and Netherland 2007, Grey et al. 2007; Bultemeier et al. 2009). Because of the pH sensitivity of these 2 active ingredients, screening species at pH 6.5 allowed for fairly long exposures to both flumioxazin and carfentrazone-ethyl (Ngim and Crosby 2001, Katagi 2003), however; the assay was developed to determine plant sensitivity and not to make field recommendations. The ability to screen a large number of species in a short period can be useful when trying to determine plant selectivity of new and emerging aquatic herbicides and decide which target and nontarget species may require further investigation on a larger-scale.

Key differences between screening terrestrial species versus submersed aquatic species is the time in which the assay has to be run, the tissue used, and the need for a dark incubation period. Results for terrestrial species can usually be obtained within hours after treatment (Vanstone and Stobbe 1977, Yanase et al. 1990, Duke and Kenyon 1993, Falk et al. 2006), whereas aquatic species required 5 d. Electrolytes for aquatic species would start to leak about 3 d after treatment, however; results were best if assays were left for 5 d (data not shown). There wasn't an increase in leakage beyond 5 d, and tubes tended to become cloudy with either bacterial or fungal contaminates. Light intensity could explain why aquatic species required 5 d compared with hours for terrestrial species. Full sunlight is required for optimal activity of protox inhibitors, such as flumioxazin and carfentrazone-ethyl (Sherman et al. 1991, Wright et al. 1995), and submersed aquatic vegetation grows in natural waters where light intensity is significantly attenuated. In a study by Mudge et al. (2012), hydrilla was exposed to 400 µg ai $L^{-1}$ flumioxazin under low (20 µmol photons $m^{-2}$ $s^{-1}$), medium (170 µmol photons $m^{-2}$ $s^{-1}$), and high light (400 µmol photons $m^{-2}$ $s^{-1}$) conditions. The time required to reduce photosynthesis by 50% was 303, 140, and 99 h for the low, medium, and high light conditions, respectively, with only the low and high light differing from each other. Increasing the light intensity in these assays could possibly shorten the time required for an accurate measurement, but further investigation is needed.

TABLE 2. MEAN (± SE) PERCENTAGE OF ELECTROLYTE LEAKAGE OF NATIVE SUBMERSED PLANTS EXPOSED TO CARFENTRAZONE-ETHYL FOR 5 D. EACH SPECIES WAS EXAMINED TWICE, AND DATA WERE POOLED. SPECIES IN ITALICS ARE INVASIVE PLANTS. EACH MEAN REPRESENTS THE AVERAGE OF 8 REPLICATE TREATMENTS. MEANS DENOTED WITH AN ASTERISK ARE SIGNIFICANTLY DIFFERENT FROM THE UNTREATED CONTROL. DATA WERE SUBJECTED TO *t* TESTS ($\alpha = 0.05$).

| Species | Control | 200 µg $L^{-1}$ |
|---|---|---|
| Not sensitive | | |
| American eelgrass | 20.0 ± 2.1 | 20.5 ± 1.7 |
| American pondweed | 44.5 ± 1.2 | 43.9 ± 1.6 |
| *Curlyleaf pondweed* | 55.4 ± 3.9 | 65.0 ± 3.9 |
| Common elodea | 28.8 ± 5.0 | 32.4 ± 5.4 |
| *Hydrilla* | 29.2 ± 1.7 | 31.9 ± 3.6 |
| Illinois pondweed | 42.7 ± 1.1 | 48.5 ± 5.0 |
| Longbeak buttercup | 46.5 ± 4.2 | 46.7 ± 2.6 |
| Southern naiad | 30.3 ± 4.0 | 40.2 ± 6.0 |
| Springtape | 24.8 ± 3.8 | 24.9 ± 3.6 |
| Waterstargrass | 18.8 ± 2.6 | 22.7 ± 4.9 |
| Sensitive | | |
| Coontail | 18.6 ± 3.0 | 92.4 ± 1.4* |
| *Eurasian watermilfoil* | 40.9 ± 3.1 | 56.4 ± 1.1* |
| Variable watermilfoil | 20.4 ± 1.1 | 66.3 ± 5.4* |

For assays with terrestrial species, leaf discs ranging from 4 to 13 mm (0.16 to 0.51 in) were used, and discs were cut to avoid the midvein (Vanstone and Stobbe 1977, Yanase et al. 1990, Duke and Kenyon 1993, Falk et al. 2006). There are numerous aquatic species, particularly submersed species, where leaf discs are not possible because of leaves being finely divided (i.e., coontail, Eurasian watermilfoil) or where the leaves are not wide enough to cut a disc without cutting a major vein (i.e., waterstargrass); therefore, apical stem sections were used instead. Terrestrial species can also have an initial "dumping" of electrolytes followed by a lag period, and to avoid that, assays are kept in the dark for a time before being exposed to light (Yanase et al. 1990, Falk et al. 2006). There is no initial "dumping" of electrolytes observed with submersed aquatic species, and no dark period was required (data not shown). Variation in electrolyte leakage is common among terrestrial species and within a species, leakage can vary depending on growing conditions and tissues examined (Duke and Kenyon 1993, Li et al. 2000). A similar variation in electrolyte leakage among species was also seen in this study (Tables 1 and 2).

The assay described here may be used to address questions about the direct effect of pH and light on the phytotoxicity of the protox-inhibiting herbicides flumioxazin and carfentrazone-ethyl. Moreover, the assay should also be tested to determine its utility for evaluating the effects of protox inhibitors on emergent and floating aquatic plant species.

## SOURCES OF MATERIALS

[1]Percival E-36L growth chamber, Percival Scientific, Inc., 505 Research Drive, Perry, IA 50220.

[2]Flumioxazin (Clipper), Valent USA Corporation, 1600 Riviera Avenue, Suite 200, Walnut Creek, CA 94596-8025.

[3]Carfentrazone-ethyl (Stingray), FMC Corporation, 1735 Market Street, Philadelphia, PA 19103.

[4]Accumet AP85 pH/conductivity meter, Fisher Scientific Company LLC, 300 Industry Drive, Pittsburgh, PA 15275-1126.

## ACKNOWLEDGEMENTS

Support for this project was provided by the Aquatic Plant Control Research Program (APCRP). The authors would like to thank Scott Western for technical assistance. Permission to publish on this information was granted by the Chief of Engineers. Citation of trade names does not constitute an official endorsement or approval of the use of such products.

## LITERATURE CITED

Bultemeier BW, Netherland MD, Ferrell JA, Haller WT. 2009. Differential herbicide response among three phenotypes of *Cabomba caroliniana*. Invasive Plant Sci. Manag. 2:352–359.

Dexter ST, Tottingham WE, Graber LF. 1932. Investigations of the hardiness of plants by measurement of electrical conductivity. Plant Physiol. 7(1):63–78.

Duke SH, Kakefuda G, Harvey TM. 1983. Differential leakage of intracellular substances from imbibing soybean seeds. Plant Physiol. 72:919–924.

Duke SO, Kenyon WH. 1993. Peroxidizing activity determined by cellular leakage, pp. 61–66. In: P. Boger and G. Sandman (*eds.*). Target assays for modern herbicides and related phytotoxic compounds. CRC, Boca Raton, FL.

Falk JS, Al-Khatib K, Peterson DE. 2006. Rapid assay evaluation of plant response to protoporphyrinogen oxidase (protox)-inhibiting herbicides. Weed Technol. 20:104–112.

Getsinger KD, Netherland MD, Grue CE, Koschnick TJ. 2008. Improvement in the use of aquatic herbicides and establishment of future research directions. J. Aquat. Plant Manage. 46:32–42.

Glomski LM, Netherland MD. 2007. Efficacy of diquat and carfentrazone-ethyl on variable-leaf milfoil. J. Aquat. Plant Manage. 45:136–138.

Glomski LM, Netherland MD. 2011. Small-scale screening of submersed aquatic plants to the herbicide topramezone. U.S. Army Engineer Research and Development Center APCRP Technical Notes Collection (ERDC/TN APCRP-CC-16), Vicksburg, MS.

Glomski LM, Poovey AG, Getsinger KD. 2006. Effect of carfentrazone-ethyl on three aquatic macrophytes. J. Aquat. Plant Manage. 44:67–69.

Grey CJ, Madsen JD, Wersal RM, Getsinger KD. 2007. Eurasian watermilfoil and parrotfeather control using carfentrazone-ethyl. J. Aquat. Plant Manage. 45:43–46.

Hess FD. 2000. Light-dependent herbicides: An overview. Weed Sci. 48(2):160–170.

Katagi T. 2003. Hydrolysis of *N*-phenylimide herbicide flumioxazin and its anilic acid derivative in aqueous solutions. J. Pestic. Sci. 28:44–50.

Kenyon WH, Duke SO, Vaughn KC. 1985. Sequence of effects of acifluorfen on physiological and ultrastructural parameters in cucumber cotyledon discs. Pestic. Biochem. Physiol. 24:240–250.

Koo SJ, Neal JC, DiTomaso JM. 1994. Quinclorac-induced electrolyte leakage in seedling grasses. Weed Sci. 42:1–7.

Koschnick TJ, Haller WT, Glasgow L. 2006. Documentation of landoltia (*Landoltia punctata*) resistance to diquat. Weed Sci. 54:615–619.

Li Z, Walker RH, Wehtje G, Hancock HG. 2000. Using electrolyte leakage to detect soybean (*Glycine max*) cultivars sensitive to sulfentrazone. Weed Technol. 14:699–704.

Mudge CR, Bultemeier BW, Haller WT. 2012. The influence of pH and light on hydrilla (*Hydrilla verticillata*) photosynthesis and chlorophyll after exposure to flumioxazin. Weed Sci. 60:4–9.

Mudge CR, Haller WT, Netherland MD, Kowalsky JK. 2010. Evaluating the influence of pH-dependent hydrolysis on the efficacy of flumioxazin for hydrilla control. J. Aquat. Plant Manage. 48:25–30.

Netherland MD, Lembi CA. 1992. Gibberellin synthesis inhibitor effects on submersed aquatic weed species. Weed Sci. 40:29–36.

Netherland MD, Getsinger KD, Stubbs D. 2005. Aquatic plant management: invasive species and chemical control. Outlooks Pest Manage. 16:100–104.

Ngim KK, Crosby DG. 2001. Fate and kinetics of carfentrazone-ethyl herbicide in California, USA, flooded rice fields. Environ. Toxicol. Chem. 20:485–490.

Nunes MES, Smith GR. 2003. Electrolyte leakage assay capable of quantifying freezing resistance in rose clover. Crop Sci. 43:1349–1357.

Senseman SA (*ed.*). 2007. Herbicide Handbook. 9th ed. Weed Science Society of America, Lawrence, KS. 458 pp.

Sherman T D, Becerril JM, Matsmoto H, Duke MV, Jacobs JM, Jacobs NJ, Duke NO. 1991. Physiological basis for differential sensitivities of plant species to protoporphyrinogen oxidase inhibitory herbicides. Plant Physiol. 97:280–287.

Sukumaran NP, Weiser CJ. 1972. An excised leaflet test for evaluating potato frost tolerance. Hortscience 7(5):467–469.

Vanstone DE, Stobbe EH. 1977. Electrolyte conductivity—A rapid measure of herbicide injury. Weed Sci. 25(4):352–354.

Wright TR, Fuerst EP, Ogg AG, Handihall UB, Lee HJ. 1995. Herbicide activity of UCC-C4243 and acifluorfen is due to inhibition of protoporphyrinogen oxidase. Weed Sci. 43:47–54.

Yanase D, Andoh A, Yasudomi N. 1990. A new simple bioassay to evaluate photosynthetic electron-transport inhibition utilizing paraquat phytotoxicity. Pestic. Biochem. Physiol. 38:92–98.