Influence of Plant Age and Size on Simazine Toxicity and Uptake

S. L. Knuteson,* T. Whitwell, and S. J. Klaine

ABSTRACT

Improper pesticide management can lead to environmental problems such as water quality degradation and ecological stress. Recent research in our laboratory has focused on development of constructed wetlands to assimilate pesticide-contaminated water. For improved aesthetics, these wetlands have been established with ornamental plant species. The effectiveness of a plant species for phytoremediation depends in part on its tolerance for the contaminant. Plant tolerance for pesticides may vary depending on plant age and size. This study examined the influence of plant age and size on the uptake, distribution, and toxicity of the herbicide simazine [2-chloro-4,6-bis(ethylamino)-1,3,5-triazine] in two ornamental wetland plants: parrot feather [*Myriophyllum aquaticum* (Vell.) Verdc.] and canna (*Canna* × *hybrida* L. 'Yellow King Humbert'). Plants of different ages and sizes were exposed to simazine in 10% Hoagland's nutrient solution. Toxicity was characterized using plant growth, water uptake, and photosynthetic yield during exposure and postexposure periods. In addition, other plants were exposed to [^{14}C] simazine in nutrient medium to characterize pesticide uptake and translocation. Four-week-old parrot feather and canna were more tolerant of simazine than two-week-old plants. The two-week-old plant tissues of both species had higher tissue burdens of simazine than four-week-old plants. Simazine was primarily accumulated in the leaves of both parrot feather and canna. These results suggest that plants in a constructed wetland designed for simazine assimilation would be more vulnerable to simazine toxicity shortly after emergence.

Extensive use of pesticides can lead to significant risk to nontarget organisms within adjacent aquatic and terrestrial ecosystems. One promising method for reducing pesticide risks may be through phytoremediation by ornamental wetland plant species. There are two main benefits of this type of remediation: economically, phytoremediation has a much lower cost than more technological remediation treatments; and, aesthetically, ornamental plants may be more attractive than classic wetland species (Cunningham et al., 1996). The result may be low-cost, low-maintenance, effective, and aesthetically pleasing constructed wetlands. Phytoremediation has been shown to be useful in the dissipation of atrazine (Alvord and Kadlec, 1996), metolachlor (Moore et al., 2001), chlorpyrophos (Moore et al., 2002), metalaxyl, simazine (Wilson, 1999), and other chlorinated compounds (Schnoor et al., 1995; Nzengung et al., 1999). Compounds with low to moderate octanol–water partition coefficients ($0.5 \geq \log K_{ow} \leq 3$) have been shown to be taken up into plants efficiently (Briggs et al., 1982; Schnoor et al., 1995). There are three main mechanisms for phytoremediation: plant uptake and sequestration of the contaminant and possible metabolites in tissue; release of root exudates to enhance biotransformation and microbial degradation; and enhanced rhizosphere mineralization (Schnoor et al., 1995).

Ongoing research in our laboratory has investigated the use of several ornamental wetland plants in constructed wetlands to remediate pesticide-contaminated rinse water generated on maintenance pads at golf courses and ornamental nurseries.

Simazine is an active ingredient in preemergent herbicides labeled for general use on turfgrasses, vegetables, orchards, and vineyards to control annual broadleaf weeds and grasses. It is also used in combination with other pesticides such as atrazine or paraquat (Crop Protection Reference, 1996). Due to its moderate water solubility, low volatility, and long soil half life, simazine may contaminate ground and surface waters (Kahn, 1978; Howard, 1991; Ahrens, 1994). Previous research has correlated simazine uptake in plants with water uptake and movement within the transpiration stream (Wilson et al., 1999). Simazine inhibits photosynthetic electron transport (Humburg et al., 1989) and is detoxified through conjugation to glutathione (GSH) by glutathione-*S*-transferase (GST) (Neuefeind et al., 1997).

For phytoremediation to be effective, the plant should be tolerant of the contaminant. Plant tolerance for herbicides may vary depending on plant age and size. Hatton et al. (1996) found that GST and GSH levels in corn decreased after 30 d of growth. Since GST is the major mechanism of detoxification for several pesticides, plant tolerance may decrease with increasing age. Other researchers found that pesticide absorption decreased as age increased, hence tolerance increased with age (Wilcut et al., 1989; Leah et al., 1995). The objective of this research was to characterize the influence of plant age and size on the toxicity, uptake, and translocation of simazine in two ornamental wetland plants: parrot feather and canna.

MATERIALS AND METHODS

Test Chemical

Simazine was chosen due to its frequent use on turfgrasses and its herbicidal effect on plants. Technical grade simazine (99.6% purity) was obtained from Novartis Crop Protection (Greensboro, NC). Because of its moderate water solubility (6.2 mg L^{-1}), low $\log K_{ow}$ (2.17), and low Henry's law constant ($K_H = 4.63 \times 10^{-9}$), simazine has the potential to contaminate

S.L. Knuteson and S.J. Klaine, Environmental Toxicology, Clemson University, P.O. Box 709, 509 Westinghouse Dr., Pendleton, SC 29670. T. Whitwell, 172 P&A, Clemson University, Clemson, SC 29634. Received 7 Nov. 2001. *Corresponding author (sknutes@clemson.edu).

Published in J. Environ. Qual. 31:2096–2103 (2002).

Abbreviations: LOEC, lowest observable effect concentration.

subsurface or surface waters through dissolution in runoff and leaching from treated soils (Kahn, 1978; Howard, 1991; Ahrens, 1994). Once in the water table, simazine has an environmental half-life of approximately 30 d in some surface waters (Kamrin, 1997). In the constructed wetlands used in ongoing research in our laboratory, initial simazine concentrations of 1.0 to 1.5 mg L^{-1} would be expected, based on moderate application rates and residual volumes within equipment to be rinsed (Wilson, 1999).

Plants

Parrot feather and canna were selected for evaluation due to their aesthetic properties and general use as ornamental plants. Parrot feather is an emergent vascular plant with feathery leaves that forms floating mats rooted in shallow waters. In constructed wetlands, these mats may reduce algal growth. Canna is an emergent macrophyte that grows 1.2 to 1.8 m tall with large foliage and produces flowers throughout the summer.

Two plant ages were evaluated: 2- and 4-wk-old plants. These groups had significantly different sizes. At time of exposure, the 2-wk-old plants were small with few roots and the 4-wk-old plants were larger with well-established root systems (Table 1).

Table 1. Plants size at the beginning of the toxicity bioassay and the uptake and translocation assessment. Data represent plant fresh weight means (with standard errors in parentheses) at the beginning of the exposure for each plant group.

	Toxicity bioassay		Uptake and translocation assessment	
Plant	2 wk old	4 wk old	2 wk old	4 wk old
	g			
Parrot feather	1.5 (0.04)	6.2 (0.15)	2.0 (0.12)	5.4 (0.29)
Canna	13.6 (0.5)	47.2 (1.1)	27.3 (0.6)	75.4 (1.0)

Toxicity Bioassay

Plant Culture Method

Stock populations of parrot feather were propagated from stem fragmentation by breaking plants into segments and allowing root formation in buckets of 10% Hoagland's nutrient solution (Hoagland and Arnon, 1938) in a glass greenhouse. Thirty plants approximately 10 cm long were clipped from the stock population and placed in darkened glass jars containing 250 mL of 10% Hoagland's liquid nutrient medium. Canna plants were propagated from rhizomes in potting soil in a glass greenhouse. Four weeks prior to exposure, 35 plants approximately 30 cm tall with three or four leaves each were detached from their rhizomes. Two weeks prior to exposure, another 35 plants approximately 10 cm tall with three or four leaves were detached from their rhizomes. All plants were placed with roots in darkened glass jars containing 300 mL of 10% Hoagland's liquid nutrient medium.

Light penetration to the roots was limited by using aluminum foil–wrapped jars and covers designed from 0.35-L (12 oz) styrofoam cups with a hole in the bottom to secure the plant with its roots submerged in medium. For the first 3 d after being detached from the rhizome, plants were placed in a humidity chamber and allowed to acclimatize to hydroponic conditions. Once removed from the humidity chamber, plants were grown 11 or 25 d, for 2- or 4-wk-old plants, respectively. Parrot feather plants were acclimatized to exposure conditions of 25 ± 2°C, 375 $\mu E\ m^{-2}\ s^{-1}$ (provided by metal halide lamps), and a 16 h light–8 h dark photoperiod. Due to size, canna were acclimatized and exposed in a greenhouse with the following conditions: a minimum light intensity of 375 $\mu E\ m^{-2}\ s^{-1}$ provided by metal halide lamps, with a maximum of approximately 1135 $\mu E\ m^{-2}\ s^{-1}$ during midday; a 16 h light–8 h dark photoperiod; and 24 ± 4°C. During acclimatization, plants were placed in fresh nutrient medium weekly. For parrot feather, medium lost due to transpiration or evaporation was replaced with deionized water; canna were given 10% Hoagland's nutrient medium due to a higher need for nutrients.

Exposure

Parrot feather and canna were exposed to 0, 0.05, 0.1, 0.5, 1.0, and 1.5 mg L^{-1} simazine and 0, 0.01, 0.5, 1.0, 1.5, and 2.0 mg L^{-1} simazine, respectively. A stock solution was prepared by dissolving technical grade simazine overnight in 10% Hoagland's nutrient medium with constant stirring. Exposure solutions were then prepared through dilution with 10% Hoagland's nutrient medium. All simazine concentrations were verified; after adjusting pH to 2.3 with 1 *M* HCl, 200 mL of each treatment solution were extracted with a solid phase extraction system (Burdick and Jackson, Muskegon, MI); simazine was eluted with 2 mL Optima grade acetone (Fisher Scientific, Fair Lawn, NJ). The acetone extract was then analyzed with a Hewlett–Packard (Palo Alto, CA) 5890 Series II gas chromatograph with FID detector and a 5-m, 0.053 ID J&W (Folsom, CA) DB-1 column with film thickness of 5 μm. All concentrations were corrected for percent recoveries, which were performed with the same procedure with a spiked 10% Hoagland's nutrient medium.

Toxicity bioassay procedures were adapted from Wilson (1999). To prevent light from reaching the root zone, exposure vessels consisted of aluminum foil–wrapped 470-mL glass containers with 0.35-L (12 oz) styrofoam cups with a hole in the bottom to hold the plant with its roots submerged in exposure solution. Individual plants were placed in exposure vessels containing 250 mL of exposure solution for parrot feather and 300 mL for canna. After 7 d, roots were rinsed with running tap water, and plants were weighed and placed in simazine-free medium for another 7 d postexposure to assess short-term recovery. Medium lost due to transpiration or evaporation was quantitatively replaced with deionized water for the parrot feather and 10% Hoagland's nutrient medium for the canna with a calibrated syringe; volumes were recorded. Evaporation controls consisted of exposure vessels with 250 mL 10% Hoagland's nutrient medium; volume was measured before and after each 7-d period.

Measured Endpoints

Toxicity of simazine was evaluated by examining the growth of all plants. All plants were measured for fresh weight prior to exposure, after the 7-d exposure period and postexposure period. Biomass production for each period was calculated from the fresh weight data. Water uptake was measured by recording the amount of medium replaced during the 7-d period and correcting for evaporation; hence, water uptake is a measure of all the water processed by the plant, including that which transpired. Chlorophyll fluorescence measurements were made on the innermost leaf of the canna on Days 0, 1, 3, 5, 7, and 13 with an OPTISCIENCES (Haverhill, MA) OS-500 modulated fluorometer. These measurements were not performed on the parrot feather due to the feathered nature of the leaves. The specific parameters measured included maximal fluorescence (F_m) and maximal steady state fluorescence (F_{ms}). These parameters were used to calculate the fluorescent yield of the plant. Fluorescent yield is a measure of the ability of the plant to transfer electrons through the photosynthetic pathway. A reduction in yield would sug-

gest that the pathway is blocked, and fewer electrons are being transferred.

Uptake and Translocation Assessment

Plant Culture Method

At 2 and 4 wk prior to exposure, 15 parrot feather and 18 canna plants were removed from stock populations. Plants were cultured and acclimatized to hydroponic conditions as in the toxicity bioassay. One week prior to exposure, plants were placed in exposure vessels and acclimatized to exposure conditions in a Conviron CMP 3244 environmentally controlled growth chamber (Controlled Environments, Pembina, ND). Conditions were held constant at 60% relative humidity, 25°C, and 375 $\mu E\ m^{-2}\ s^{-1}$ from fluorescent and incandescent lamps on a 16 h light–8 h dark photoperiod.

Exposure

[^{14}C] Simazine, radiolabeled at the 2-, 4-, and 6-triazine ring positions, was used to characterize the uptake and translocation of simazine throughout the plants. It had a chemical purity of 99.3%, a radiopurity of 98.7%, a specific activity of 7.4×10^7 Bq mg^{-1}, and was obtained from Novartis Crop Protection. Exposure solution consisted of 0.12 mg L^{-1} (8.4×10^6 Bq L^{-1}) and 0.90 mg L^{-1} (6.6×10^7 Bq L^{-1}) [^{14}C] simazine in 10% Hoagland's nutrient medium, for parrot feather and canna, respectively. Uptake and translocation methods were adapted from Wilson (1999).

Individual plants were exposed to 250 mL of treatment solution. For parrot feather, exposure vessels consisted of 250-mL (500-mL for canna) sidearm vacuum flasks with one-way valves to prevent gas movement out of the flask assembly. Number 6 silicon stoppers were used, along with Qubitac putty (Qubit, Kingston, ON, Canada) to seal plants with roots submerged in exposure solution. Flasks were fitted with [^{14}C] CO_2 (mineralization) and VO [^{14}C] (volatilization) traps via a teflon tube with a one-way valve through a small hole in the stopper allowing air flow out of the flask through the traps. The first trap contained 15 mL of 0.5 *M* NaOH for [^{14}C] CO_2 retention and the second, 4 g of activated charcoal (Sigma, St. Louis, MO) for VO [^{14}C]. Two chemical reference vessels, without plants but containing exposure solution, and two control vessels, with plants in simazine-free medium, were also prepared as described above and handled as the exposure vessels.

With a 60-mL syringe attached to the one-way valve on the sidearm of the flask, all air in the vessels was purged through the traps daily. Medium lost due to transpiration was quantitatively replaced with deionized water for parrot feather and 10% Hoagland's nutrient medium for canna with a calibrated syringe. The amount lost was recorded daily.

Three exposed plants were randomly selected on Days 1, 3, 5, and 7. Roots were rinsed with running tap water for one minute and blotted dry. Plants were weighed and dissected into leaves, stems, roots, rhizomes, and flowers, depending on species. Plant parts were stored in aluminum foil at −80°C until analysis.

Tissue Analysis

Prior to analysis, all tissues were freeze-dried and weighed. Tissues were combusted with an R.J. Harvey (Hillsdale, NJ) biological oxidizer at 900°C for 3 min. The [^{14}C] CO_2 formed during combustion was captured in R.J. Harvey [^{14}C] CO_2 trapping cocktail. Cocktail was then analyzed for [^{14}C] CO_2 content with a Beckman (Fullerton, CA) LS 6500 liquid scintillation counter. The disintegrations per minute (DPM) mode was used to count each sample for 10 min.

Solution Analysis

Exposure solution was analyzed before and after treatment of plants for [^{14}C] simazine content by placing 0.2 mL of solution in 6 mL of Scinti Verse, I or BD, scintillation cocktail (Fisher Scientific). Samples were counted for 3 min with the Beckman LS 6500 liquid scintillation counter in DPM mode.

Endpoints

The [^{14}C] activity found in each part of the plant and solution was used to determine the percentage of the initial [^{14}C] activity found distributed throughout the plant and solution at the end of the exposure. The tissue burden of the plant was calculated by dividing the total [^{14}C] simazine found in the tissue by its dry weight.

Statistics

A completely randomized design was used with four replicates per treatment for the toxicity bioassay and three replicates for the uptake and translocation assessment. However, during the toxicity bioassay of the canna, five 4-wk-old plants flowered. One plant from each of the control, 0.01, 0.5, 1.5, and 2.0 mg L^{-1} simazine exposures was removed from analysis. Therefore, the number of replicates for the 4-wk-old canna was three for these treatments. Biomass production, water

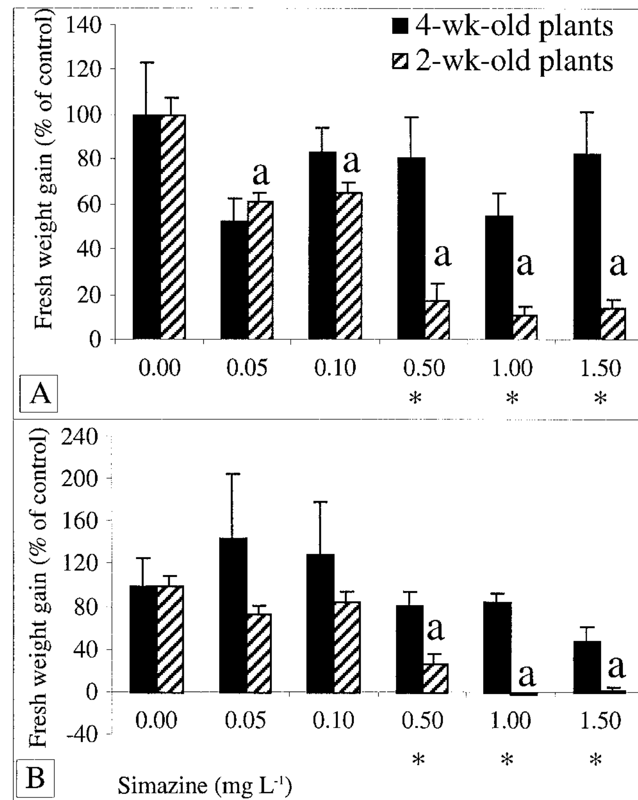

Fig. 1. Biomass production of parrot feather relative to the control during (*A*) a 7-d exposure period and (*B*) a 7-d postexposure period to simazine. A * indicates that the effect of simazine on 4-wk-old plants is significantly different from that of 2-wk-old plants, while the letter *a* indicates that the 2-wk-old treatment is statistically different from the control. $P < 0.05$, $n = 4$, bars represent standard error.

uptake, and fluorescence data were ranked with the Wilcoxon rank-sum test. Data were analyzed with SAS 6.12 for MacIntosh (SAS Institute, 1989). Differences were determined with analysis of variance (ANOVA), Dunnett's test, and repeated measures programs at $P < 0.05$.

RESULTS

Toxicity Bioassay

Parrot Feather

Biomass production. Biomass production of 2-wk-old parrot feather during the 7-d exposure period was significantly reduced relative to control at simazine exposures of 0.05 mg L^{-1} and greater (Fig. 1A). While the lowest observable effect concentration (LOEC) for 2-wk-old plants was 0.05 mg L^{-1} simazine, no statistically significant effect on biomass production of 4-wk-old parrot feather was seen at any concentration. Biomass production by 2-wk-old parrot feather was significantly less than that of 4-wk-old plants at 0.5, 1.0, and 1.5 mg L^{-1} simazine.

When placed in simazine-free medium for 7 d, 2-wk-old plants exposed to 0.05 and 0.1 mg L^{-1} simazine recovered completely, those exposed to 0.5 mg L^{-1} simazine showed no recovery, and those exposed to 1.0 and 1.5 mg L^{-1} simazine senesced and biomass was reduced further (Fig. 1B).

Water uptake. Water uptake by 2-wk-old parrot feather during the 7-d exposure period, normalized to the plant weight at the beginning of the exposure, was significantly reduced relative to control at 0.5, 1.0, and 1.5 mg L^{-1} simazine (Fig. 2A). While the LOEC for the weight-normalized water uptake by the 2-wk-old plants was 0.5 mg L^{-1} simazine, no effect on water uptake by the 4-wk-old parrot feather was seen at any concentration tested. As seen in Fig. 2B, water use efficiency, represented here by the amount of water needed to produce a gram of plant, decreased for the 2-wk-old plants, with a LOEC of 0.5 mg L^{-1} simazine. However, no differences were seen for the 4-wk-old plants.

Canna

Biomass production. Biomass production by 2-wk-old canna during a 7-d exposure to simazine (Fig. 3A) was significantly reduced relative to control at exposures greater than 1.0 mg L^{-1}. The LOEC for biomass production by the 2-wk-old plants was 1.5 mg L^{-1} simazine. Biomass production of 4-wk-old canna was not significantly affected by the simazine concentrations used. Biomass production for 2-wk-old canna was significantly less than that for 4-wk-old plants. Affected 2-wk-old plants showed incomplete recovery in biomass production after 7 d in simazine-free medium (Fig. 3B).

Water uptake. Water uptake by 2-wk-old canna during the 7-d exposure period, normalized to the plant weight

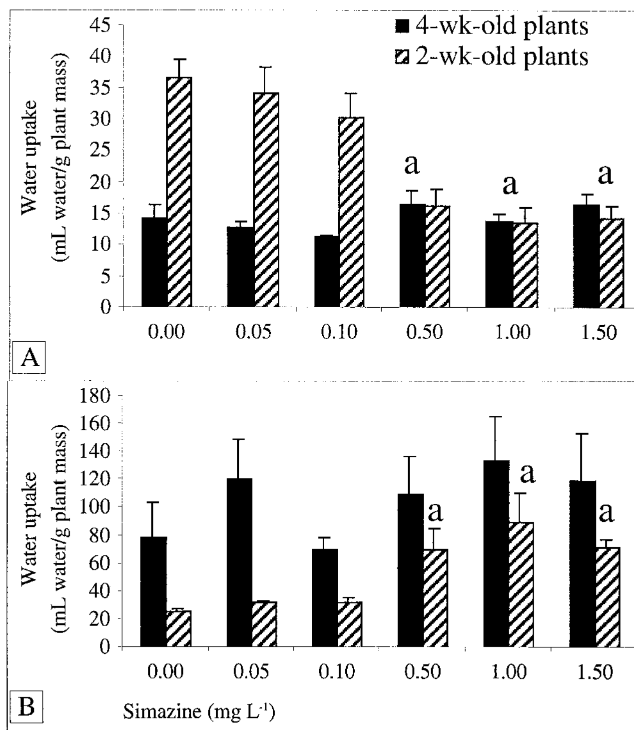

Fig. 2. Weight normalized water uptake by parrot feather relative to control during a 7-d exposure to simazine. (*A*) Water uptake normalized to beginning plant weight. (*B*) Water uptake normalized to biomass gain during the 7-d exposure to simazine. A * indicates that the effect of simazine on 4-wk-old plants is significantly different from that of 2-wk-old plants, while the letter *a* indicates that the treatment is statistically different from the control. $P < 0.05$, $n = 4$, bars represent standard error.

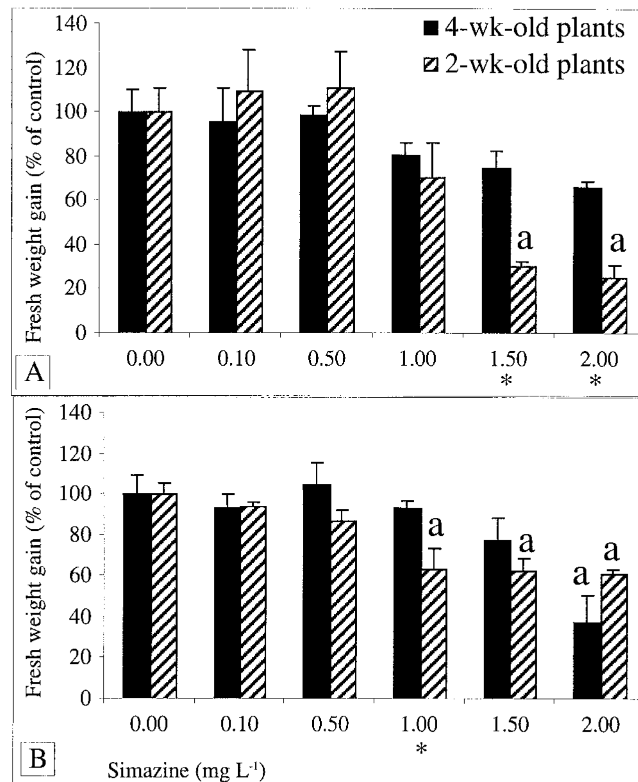

Fig. 3. Biomass production of canna relative to the control during (*A*) a 7-d exposure period and (*B*) a 7-d postexposure period to simazine. A * indicates that the effect of simazine on 4-wk-old plants is significantly different from that of 2-wk-old plants, while the letter *a* indicates that the treatment is statistically different from the control. $P < 0.05$, bars represent standard error.

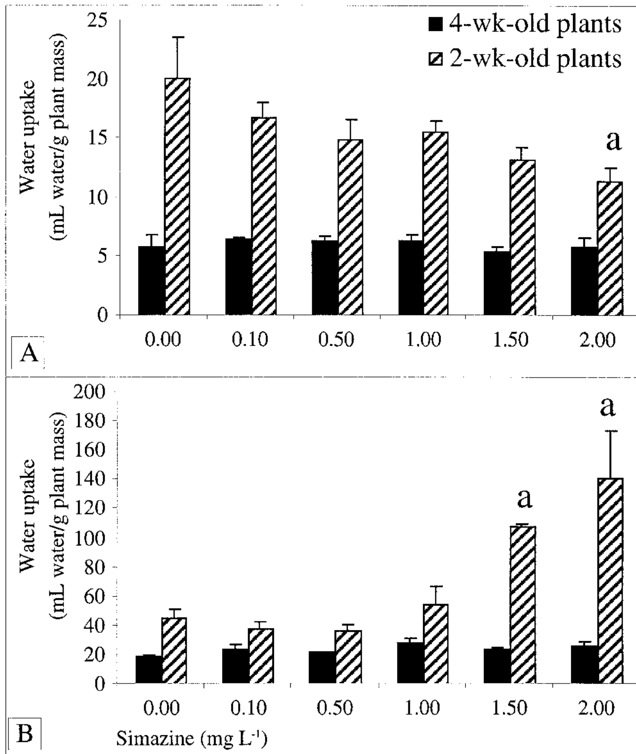

Fig. 4. Water uptake by canna relative to control during a 7-d exposure to simazine. (*A*) Water uptake normalized to beginning plant weight. (*B*) Water uptake normalized to biomass gain during the 7-d exposure to simazine. A * indicates that the effect of simazine on 4-wk-old plants is significantly different from that of 2-wk-old plants, while the letter *a* indicates that the treatment is statistically different from the control. $P < 0.05$, bars represent standard error.

at the beginning of the exposure (Fig. 4A), was significantly reduced relative to control in the 2.0 mg L^{-1} simazine exposure. While the LOEC for the weight-normalized water uptake by the 2-wk-old plants was 1.5 mg L^{-1} simazine, weight-normalized water uptake by 4-wk-old canna during the exposure period was not significantly affected in any treatment tested. As seen in Fig. 4B, water use efficiency decreased for the 2-wk-

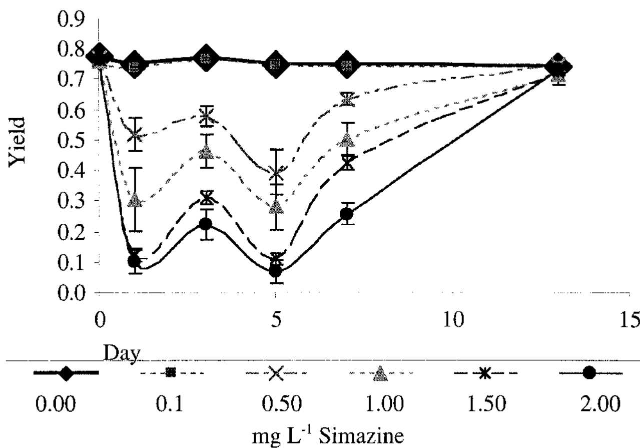

Fig. 5. Fluorescent yield by 2-wk-old canna during a 7-d exposure period and a 7-d postexposure period to simazine. Bars represent standard error.

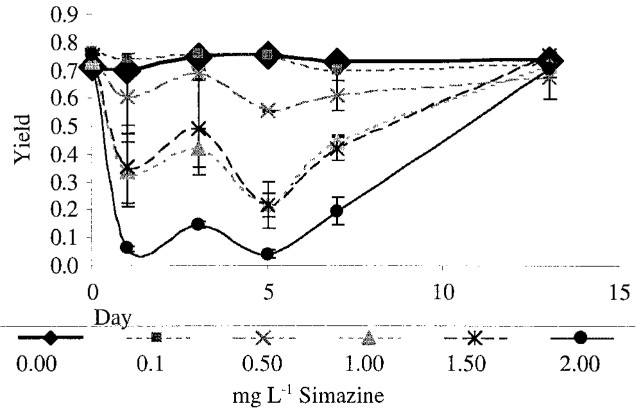

Fig. 6. Fluorescent yield by 4-wk-old canna during a 7-d exposure period and a 7-d postexposure period to simazine. Bars represent standard error.

old plants, with a LOEC of 1.0 mg L^{-1} simazine. However, no differences were seen for the 4-wk-old plants.

Fluorescent yield. Fluorescent yield of 2- and 4-wk-old canna during the 7-d exposure to simazine was significantly reduced relative to control at exposures of 0.5 and 1.0 mg L^{-1}, respectively (Fig. 5 and 6). After 7 d in simazine-free medium, however, all plants fully recovered. By Day 1 of the simazine exposure (Fig. 7), fluorescent yield of the 2-wk-old canna was reduced to 41, 17, and 14% of controls by the 1.0, 1.5, and 2.0 mg L^{-1} exposures, respectively. Fluorescent yield of the 4-wk-old canna was reduced to 48, 51, and 8% of controls by the 1.0, 1.5, and 2.0 mg L^{-1} exposures, respectively. The LOEC for the 2- and 4-wk-old plants on Day 1 of the simazine exposure was 0.5 and 1.0 mg L^{-1}, respectively; although there was no significant difference between the reduction in yield by the 2- and 4-wk-old plants. On Day 3 of the simazine exposure (Fig. 5 and 6), there was a general increase in yield for both 2- and 4-wk-old plants. Although it was not significant, this was probably due to increased temperatures that day.

Uptake and Translocation Assessment

Parrot Feather

The [^{14}C] simazine dissipation from exposure solution during the 1-wk exposure was almost 50% for 2-wk-old

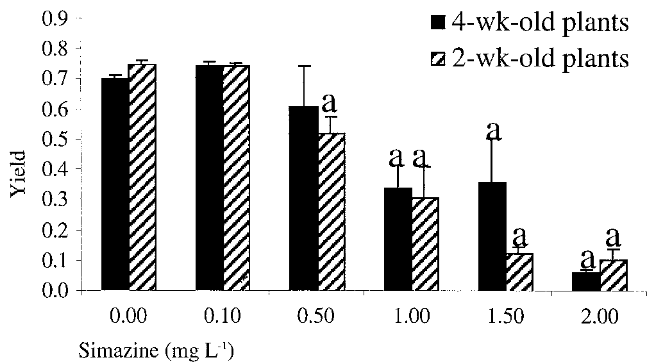

Fig. 7. Fluorescent yield by 2- and 4-wk-old canna on Day 1 of the 7-d exposure to simazine. A * indicates that the effect of simazine on 4-wk-old plants is significantly different from that of 2-wk-old plants, while the letter *a* indicates that the treatment is statistically different from the control. $P < 0.05$, bars represent standard error.

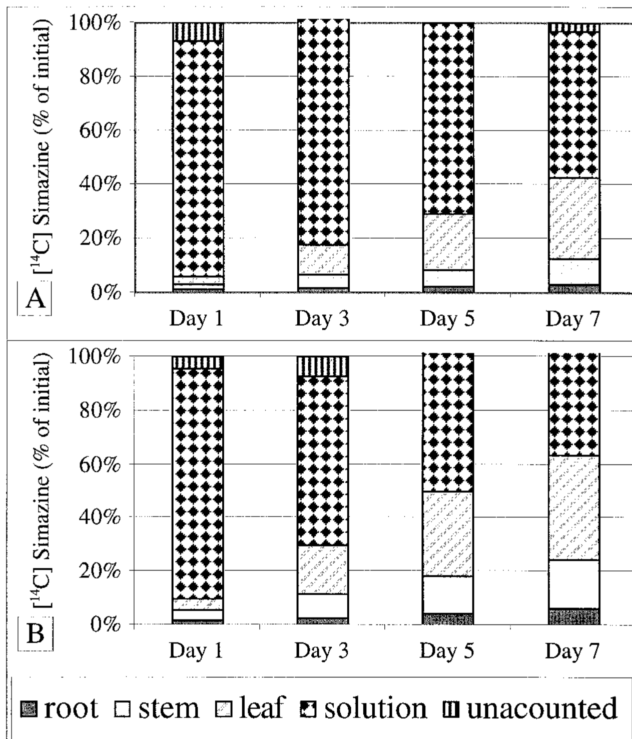

Fig. 8. Distribution of [14C] simazine in 2- and 4-wk-old parrot feather tissues during a 7-d exposure. (*A*) 2-wk-old plants. (*B*) 4-wk-old plants. Data are presented as percent of initial activity. See Table 2 for actual mean and standard error.

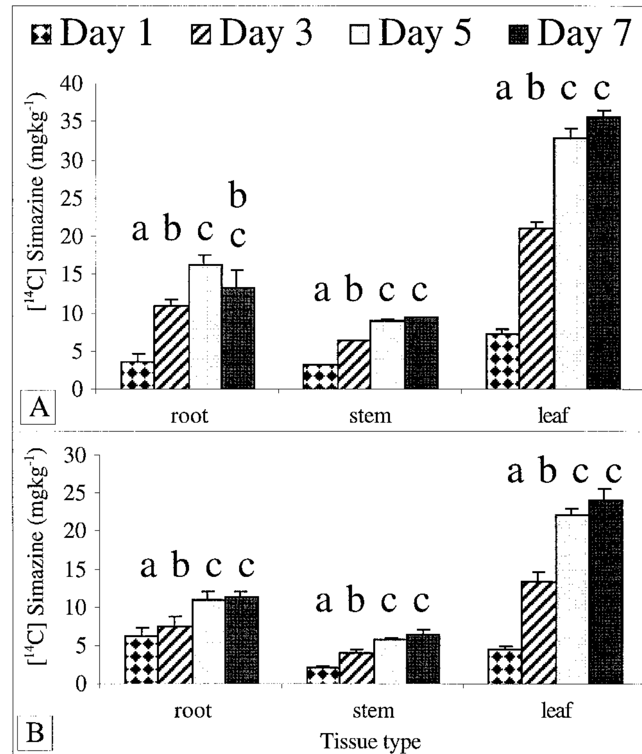

Fig. 9. The [14C] simazine tissue burden of 2- and 4-wk-old parrot feather during a 7-d exposure. (*A*) 2-wk-old plants. (*B*) 4-wk-old plants. Letters represent statistically different groupings, $n = 3$, $P < 0.05$, bars represent standard error.

plants and greater than 60% for 4-wk-old plants (Fig. 8, Table 2). Although most of the activity removed from solution was measured throughout the plants, up to 7% of the initial activity was left unaccounted for. The activity that was unaccounted for could have been lost when the roots were rinsed. The [14C] CO_2 (mineralization) and VO [14C] (volatilization) traps did not contain significant amounts of activity, suggesting minimal loss from solution duet to mineralization or volatilization.

The [14C] simazine uptake into the plant was correlated with total water taken into the plant during exposure for both 2- and 4-wk-old parrot feather ($R^2 = 0.930$ and 0.928, respectively). The [14C] activity was distributed throughout the plant but accumulated mainly in the leaves of both 2- and 4-wk-old parrot feather. Activity in all tissues increased with time. The tissue of 4-wk-old plants accumulated [14C] activity at a significantly higher percentage than the 2-wk-old plants.

The tissue burden (mg [14C] simazine kg^{-1} dry weight of tissue) of parrot feather tissues (Fig. 9) increased over Days 1 through 5, but was not significantly different on Days 5 and 7. On Day 7, the tissue burden of 2-wk-old parrot feather tissues was significantly higher than that of the 4-wk-old plants.

Table 2. Distribution of [14C] simazine in 2- and 4-wk-old parrot feather during a 7-d exposure.

Plant part	Day 1	Day 3	Day 5	Day 7
	% of initial activity			
	2-wk-old plants			
Root	0.9 (0.1)†	1.6 (0.1)	2.2 (0.0)	2.9 (0.3)
Stem	2.3 (0.3)	4.9 (0.5)	6.4 (0.4)	9.6 (1.6)
Leaf	3.1 (0.4)	11.3 (1.8)	20.2 (1.4)	29.8 (3.0)
Solution	86.8 (2.7)	89.0 (5.8)	70.9 (2.7)	54.8 (4.3)
Unaccounted	6.9 (2.5)	–	1.5 (0.3)	2.9 (0.9)
	4-wk-old plants			
Root	1.7 (0.2)	2.7 (0.3)	4.5 (1.0)	6.0 (0.5)
Stem	3.7 (0.4)	9.0 (0.8)	13.8 (1.2)	18.4 (1.0)
Leaf	4.7 (0.5)	17.9 (0.9)	31.6 (4.1)	38.8 (1.6)
Solution	85.4 (1.2)	62.9 (0.4)	63.2 (2.9)	39.0 (2.6)
Unaccounted	4.5 (0.3)	7.5 (1.3)	–	–

† Data are the mean values, with standard errors in parentheses.

Canna

The [14C] simazine dissipation from exposure solution during the 1-wk exposure was greater than 70% for 2-wk-old plants and greater than 80% for 4-wk-old plants (Fig. 10, Table 3). Although most of the activity removed from solution was measured throughout the plants, up to 26% of the initial activity was left unaccounted for. The activity that was unaccounted for could have been lost when the roots were rinsed. The [14C] CO_2 (mineralization) and VO [14C] (volatilization) traps did not contain significant amounts of activity.

The [14C] simazine uptake into the plant was correlated with total water taken into the plant during exposure for both 2- and 4-wk-old canna ($R^2 = 0.877$ and 0.940, respectively). The [14C] activity was distributed throughout the plant but accumulated mainly in the leaves of both 2- and 4-wk-old canna; however, the percentage of activity in the roots, rhizome, and stem remained constant. This suggests that the activity found

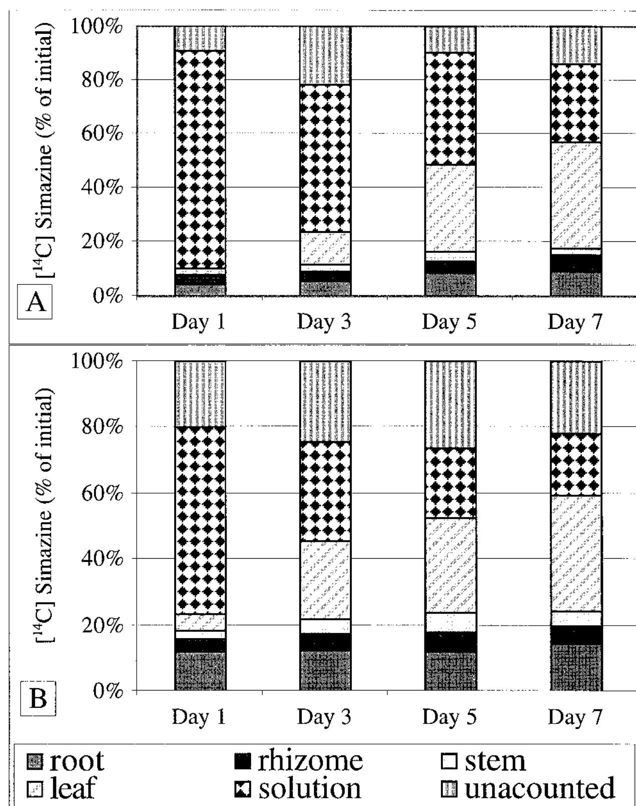

Fig. 10. Distribution of [^{14}C] simazine in 2- and 4-wk-old canna tissues during a 7-d exposure. (*A*) 2-wk-old plants. (*B*) 4-wk-old plants. Data are presented as percent of initial activity. See Table 3 for actual means and standard error.

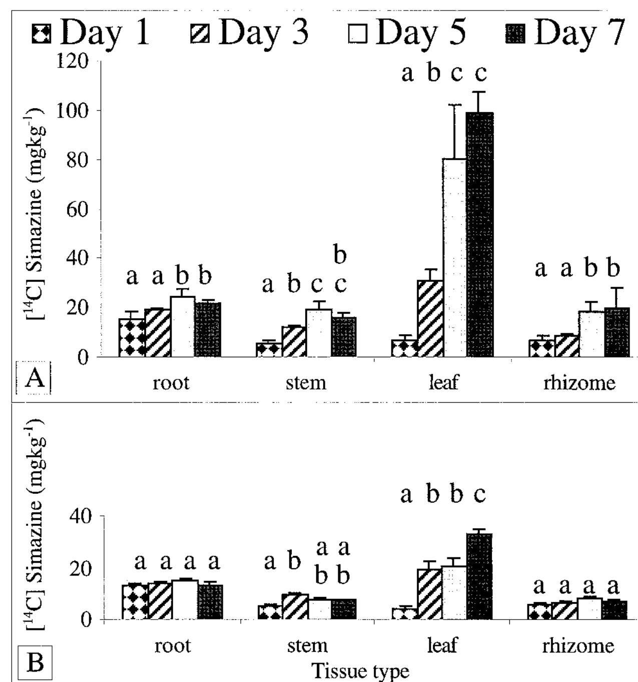

Fig. 11. The [^{14}C] simazine tissue burden of 2- and 4-wk-old canna during a 7-d exposure. (*A*) 2-wk-old plants. (*B*) 4-wk-old plants. Letters represent statistically different groupings, $n = 3$, $P < 0.05$, bars represent standard error.

in these tissues is due to translocation, rather than accumulation. The primary sink for [^{14}C] activity was the leaves for both 2- and 4-wk-old canna. By Day 7, there was no significant difference in the activity accumulated by 2- and 4-wk-old canna leaves (Fig. 10). There was a significantly higher percentage of activity in the roots of 4-wk-old plants as compared with 2-wk-old plants.

The tissue burden of canna (Fig. 11) tissues remained constant in root, rhizome, and stem of both 2- and 4-wk-old plants. The tissue burden of the 2-wk-old tissues, however, is significantly higher than that of the 4-wk-old tissues. On Day 7, the tissue burden of the leaves of the 2-wk-old canna was three times that of 4-wk-old plants.

Table 3. Distribution of [^{14}C] simazine in 2- and 4-wk-old canna during a 7-d exposure.

Plant part	Day 1	Day 3	Day 5	Day 7
	% of initial activity			
	2-wk-old plants			
Root	4.4 (0.9)†	5.6 (0.8)	8.7 (0.7)	8.9 (0.9)
Stem	2.7 (0.2)	3.8 (0.2)	4.3 (0.2)	6.0 (1.3)
Leaf	0.8 (0.2)	2.2 (0.3)	3.3 (0.2)	2.5 (0.3)
Solution	2.6 (0.7)	12.1 (1.4)	32.2 (6.1)	39.5 (5.3)
Unaccounted	80.7 (7.1)	54.6 (1.0)	41.8 (5.0)	29.3 (4.3)
	4-wk-old plants			
Root	11.7 (0.9)	12.3 (2.2)	11.8 (1.5)	14.4 (0.3)
Stem	3.9 (0.5)	4.9 (0.8)	5.9 (2.6)	5.1 (0.7)
Leaf	2.7 (0.4)	4.6 (0.8)	6.1 (0.7)	4.5 (0.5)
Solution	5.1 (2.6)	23.8 (4.1)	28.7 (16.5)	35.4 (3.7)
Unaccounted	57.1 (3.1)	30.2 (2.7)	21.1 (6.5)	18.9 (3.3)

† Data are the mean values, with standard errors in parentheses.

DISCUSSION

The results of the toxicity bioassay demonstrated that 4-wk-old parrot feather and canna were more tolerant of simazine than 2-wk-old plants. However, 4-wk-old plants took up more water and [^{14}C] simazine than 2-wk-old plants. The [^{14}C] simazine accumulated primarily in the leaves of both parrot feather and canna. The tissue burden of [^{14}C] simazine in the leaves of the 2-wk-old parrot feather and canna was 1.5 and 3.0 times that of 4-wk-old plants, respectively. Hence, while 4-wk-old plants took up more simazine than 2-wk-old plants, the tissue burden, normalized for plant biomass, was less for the 4-wk-old plants. The increased tolerance might simply be a function of dilution of the simazine in more biomass, reducing the effective concentration at the active site.

Simazine can be metabolized to dealkylated simazine and hydroxy-simazine and stored in the vacuoles; however, the main form of detoxification is through conjugation to glutathione (GSH) using glutathione-*S*-transferases (GST). The foliage of plants has the highest levels of GSH and GST (Castelfranco et al., 1961; Beynon et al., 1972; Akinyemiju et al., 1983; Neuefeind et al., 1997). Hatton et al. (1996) demonstrated that the levels of glutathione in corn plants declined after 30 d of growth. Plant tolerance for atrazine, however, increased with age. Similarly, parrot feather and canna tolerance for simazine, in this study, increased with plant age and size.

The majority of the [^{14}C] activity removed from the exposure solution in the uptake and translocation assessment was found distributed throughout the plants; however, 7 and 26% of the activity was not measured for parrot feather and canna, respectively. The [^{14}C] CO_2 (mineralization) and VO [^{14}C] (volatilization) traps did not contain significant amounts of activity. Shone and Wood (1972) reported that barley, exposed to [^{14}C] simazine, did not mineralize the pesticide. Since chemical reference controls showed no disappearance of activity, the missing activity in the present work was probably lost when roots were rinsed, or the plants were oxidized.

Within 24 h, photosynthesis in the canna was reduced to less than 10% of normal for both the 2- and 4-wk-old plants exposed to 2.0 mg L^{-1} simazine. Once the plants were removed from the simazine, however, photosynthesis recovered to normal levels in all the canna. This suggests that if 4-wk-old plants were exposed for a longer period, growth may have been reduced in these plants as well. The photosynthetic endpoint was more sensitive than growth. However, it also illustrated that the effects of simazine were reversible. Hence, if the plant does not senesce, it will probably recover and continue to grow once the simazine burden is removed.

Macrophyte toxicity tests generally use duckweed (*Lemna* spp.), but some use plants similar in size to the 2-wk-old plants of this study (Powell et al., 1996; Wilson et al., 1999). Other toxicity tests use seed germination and seedling growth as endpoints (Wang and Kenturi, 1990; Walsh et al., 1991; Wang, 1992). The results of these studies might not be representative of macrophytes in the aquatic environment if the exposure is at a different life stage. In the environment, plants can vary in size depending on plant life stage, water and nutrient availability, and other environmental conditions. The tolerance of plants may depend on the size of the plant, as shown in this study. Consideration of plant size must be taken when extrapolating results from studies with macrophytes.

A constructed wetland designed for assimilation of contaminants may be used year round; however, the plants will vary in size throughout the year. Should the constructed wetland be used when plants are not full size? If they are used when the plants are young, growth may be reduced. These results suggest that plants in a constructed wetland designed for simazine assimilation would be more vulnerable to simazine toxicity shortly after emergence.

REFERENCES

Ahrens, W.H. 1994. Herbicide handbook of the Weed Science Society of America. WSSA, Champaign, IL.

Akinyemiju, O.A., D.I. Dickmann, and R.A. Leavitt. 1983. Distribution and metabolism of simazine in simazine-tolerant and -intolerant poplar (*Populus* sp.) clones. Weed Sci. 31:775–778.

Alvord, H.H., and R.H. Kadlec. 1996. Atrazine fate and transport in the Des Plaines wetlands. Ecol. Modell. 90:97–107.

Beynon, K.I., G. Stoydin, and A.N. Wright. 1972. A comparison of the breakdown of the triazine herbicides cyanazine, atrazine and simazine in soils and in maize. Pestic. Biochem. Physiol. 2:153–161.

Briggs, G.G., R.H. Bromilow, and A.A. Evans. 1982. Relationships between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. Pestic. Sci. 13:495–503.

Castelfranco, P., C.L. Roy, and D.B. Deutsch. 1961. Non-enzymatic detoxification of 2-chloro-4,6-bis(ethylamino)-s-triazine (simazine)by extracts of *Zea mays*. Weeds 9:580–591.

Crop Protection Reference. 1996. Crop protection reference. C & P Press, New York.

Cunningham, S.C., T.A. Anderson, A.P. Schwab, and F.C. Hsu. 1996. Phytoremediation of soils contaminated with organic pollutants. Adv. Agron. 56:55–114.

Hatton, P.J., D.J. Cole, and R. Edwards. 1996. Influence of plant age on glutathione levels and glutathione transferases involved in herbicide detoxification in corn (*Zea mays* L.) and giant foxtail (*Setaria faberi* Herm). Pestic. Biochem. Physiol. 54:199–209.

Hoagland, D.R., and D.I. Arnon. 1938. The water culture method for growing plants without soil. Circ. 347. Univ. of California, College of Agric., Agric. Exp. Stn., Berkeley.

Howard, P.H. 1991. Handbook of environmental fate and exposure data for organic chemicals. Lewis Publ., Chelsea, MI.

Humburg, N., S. Colby, R. Lynn, E. Hill, W. McAvoy, L. Kitchen, and R. Prasad. 1989. Herbicide handbook of the Weed Science Society of America. WSSA, Champaign, IL.

Kahn, S.U. 1978. Kinetics of hydrolysis of atrazine in aqueous fulvic acid solution. Pestic. Sci. 9:39–43.

Kamrin, M.A. ed. 1997. Pesticide profiles: Toxicity, environmental impact, and fate. CRC Press, Boca Raton, FL.

Leah, J.M., J.C. Caseley, C.R. Riches, and B. Valverde. 1995. Age-related mechanisms of propanil tolerance in jungle-rice, *Echinochloa colona*. Pestic. Sci. 43:347–354.

Moore, M.T., J.H. Rodgers, S. Smith, and C.M. Cooper. 2001. Mitigation of metolachlor-associated agricultural runoff using constructed wetlands in Mississippi, USA. Agric. Ecosyst. Environ. 84:169–176.

Moore, M.T., R. Schulz, C.M. Cooper, S. Smith, and J.H. Rodgers. 2002. Mitigation of chlorpyrofos runoff using constructed wetlands. Chemosphere 46:827–835.

Neuefeind, T., P. Reinemer, and B. Bieseler. 1997. Plant glutathione S-transferases and herbicide detoxification. Biol. Chem. 378:199–205.

Nzengung, V.A., L.N. Wolfe, D.E. Rennels, S.C. McCutceon, and C. Wang. 1999. Use of aquatic plants and algae for decontamination of waters polluted with chlorinated alkanes. Int. J. Phytorem. 1:203–226.

Powell, R.L., R.A. Kimerle, and E.M. Moser. 1996. Development of a plant bioassay to assess toxicity of chemical stressors to emergent macrophytes. Environ. Toxicol. Chem. 15:1570–1576.

SAS Institute. 1989. SAS user's guide. SAS Inst., Cary, NC.

Schnoor, J.L., L.A. Licht, S.C. McCutcheon, N.L. Wolfe, and L.H. Carreira. 1995. Phytoremediation of organic and nutrient contaminants. Environ. Sci. Technol. 29:318A–323A.

Shone, M.G.T., and A.V. Wood. 1972. Factors affecting absorption and translocation of simazine by barley. J. Exp. Bot. 23:141–151.

Walsh, G.E., D.E. Weber, T. Simon, and L.K. Brashers. 1991. Toxicity tests of effluents with marsh plants in water and sediment. Environ. Toxicol. Chem. 10:517–523.

Wang, W. 1992. Use of plants for the assessment of environmental contaminants. Rev. Environ. Contam. Toxicol. 126:87–96.

Wang, W., and P. Kenturi. 1990. Comparative seed germination tests using ten plant species for assessing effluent toxicity from a metal engraving sample. Water Air Soil Pollut. 52:369–375.

Wilcut, J.W., G.R. Wehtje, M.G. Patterson, T.A. Cole, and T.V. Hicks. 1989. Absorption, translocation, and metabolism of foliar-applied chlorimuron in soybeans (*Glycine max*), peanuts (*Arachis hypogaea*), and selected weeds. Weed Sci. 37:175–180.

Wilson, P.C. 1999. Phytoremediation of metalaxyl and simazine residues in water using ornamental and native wetland plant species. Ph.D. diss. Clemson Univ., Clemson, SC.

Wilson, P.C., T. Whitwell, and S.J. Klaine. 1999. Phytotoxicity, uptake, and distribution of [14C] simazine in *Canna hybrida* 'Yellow King Humbert'. Environ. Toxicol. Chem. 18:1462–1468.