

Phytotoxicity, uptake, and distribution of ^{14}C -simazine in *Acorus gramenius* and *Pontederia cordata*

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Phytoremediation depends on the ability of plants to tolerate and assimilate contaminants. We have been interested in the use of common ornamental plants to ameliorate the effects of pesticide waste on golf courses and ornamental plant nurseries. This research characterized the interaction between two ornamentals, *Acorus gramenius* and *Pontederia cordata*, and the herbicide simazine. Simazine tolerance levels for the ornamentals were determined by exposing plants to 0, 0.01, 0.03, 0.1, 0.3, 1.0, or 3.0 mg L⁻¹ in aqueous nutrient media for 7 d. Response endpoints included fresh weight gains for both species after 7 d exposure and 7 d postexposure. Quantum efficiency of *P. cordata* was also measured using dark-adapted (F_v/F_m) and light-adapted (fluorescence yields) plants. Simazine uptake and distribution within each plant was determined by exposing plants to [^{14}C]-simazine in nutrient media (0.24 mg L⁻¹) for 1, 3, 5, or 7 d. Plant tissues were combusted and analyzed by liquid scintillation counting. *Pontederia cordata* fresh weight gains were reduced 76 and 70% by 1.0 and 3.0 mg L⁻¹, respectively, after 7 d exposure. *Acorus gramenius* fresh weight gains were reduced 103, 124, and 144% at 0.3, 1.0, and 3.0 mg L⁻¹ following 7 d exposure, respectively. Photosynthetic efficiency measured on dark-adapted *P. cordata* (F_v/F_m) was reduced 21, 47, and 71% by 0.3, 1.0, and 3.0 mg L⁻¹, respectively, whereas photosynthetic efficiency measured on light-adapted plants (fluorescence yields) were reduced 9, 25, 59, 87, and 96%, respectively, by 0.03, 0.1, 0.3, 1, and 3 mg L⁻¹. Simazine activity in solution was reduced 45 and 34% over 7 d with *A. gramenius* and *P. cordata*, respectively. By day 7, activity was distributed throughout the plants, but predominantly in the leaves. Uptake of simazine was correlated with water uptake throughout the 7 d. These results suggest that *A. gramenius* and *P. cordata* may be good candidates for incorporation in a phytoremediation scheme for simazine.

Nomenclature: Simazine; *Acorus gramenius* L., sweet flag; *Pontederia cordata* L., pickerel weed.

Key words: Phytoremediation, triazine.

Simazine is the herbicidal active ingredient of commercial formulations of Princep (Novartis Crop Protection, Greensboro, NC, USA). This herbicide is moderately soluble in water (6.2 mg L⁻¹ @ 22 C) and is not volatile (vapor pressure: 1.5×10^{-8} mm Hg @ 25 C) (Anonymous 1995a). Simazine inhibits photosynthetic electron transport by binding to the plastoquinone B (Q_B) protein binding site on the D1 protein of the photosystem II (PSII) complex in chloroplast thylakoid membranes (Ahrens 1994). The bound simazine prevents transfer of electrons from Q_A to Q_B , thus breaching photosynthetic electron transport, CO₂ fixation, and the production of ATP and NADPH₂. However, plant death most commonly results from subsequent lipid peroxidation caused by triplet-state chlorophyll and singlet oxygen, common products resulting from the inability to reoxidize Q_A (Ahrens 1994).

Commercial formulations of simazine are labeled for use in the agricultural, turfgrass, and nursery industries. These industries may generate significant amounts of simazine-contaminated water by the common practice of rinsing sprayers after use. Most manufacturers recommend that the rinsates be applied to areas that are normally treated. Improper disposal of these contaminated rinsates may result in significantly altered nontarget ecosystems due to simazine's effectiveness as a photosynthetic inhibitor. Photosynthesis

was inhibited 50% in filamentous algae at 0.22 to 0.95 mg L⁻¹ and at 0.95 mg L⁻¹ in nonfilamentous algae (O'Neal and Lembi 1983). Likewise, *Myriophyllum verticillatum* L. (whorled watermilfoil), *Potamogeton pectinatus* L. (sago pondweed), and *Vaucheria dichotoma* (L.) AG. senesced at simazine concentrations of 1 mg L⁻¹, whereas *Rhizoclonium heiroglyphicum* and *Cladophora glomerata* senesced at 0.5 mg L⁻¹ (Fowler 1977). Transient alterations in aquatic invertebrate community composition due to simazine concentrations of up to 0.5 mg L⁻¹ have also been reported (Ellis et al. 1976).

Phytoremediation offers one possible method for removing simazine from contaminated water. Phytoremediation is the use of plants and plant growth as a technique for detoxifying environmental sites contaminated with organic and inorganic pollutants. This technology exploits the ability of plants to extract or mineralize xenobiotics in the surrounding environment, as well as the tolerance of these plants to the contaminants. According to Schnoor et al. (1995), this developing technology is best suited for sites with shallow contamination (< 5 m deep), moderately hydrophobic pollutants ($\log K_{ow} = 0.5$ to 3), short-chain aliphatic chemicals, and excess nutrients. The $\log K_{ow}$ for simazine is 2.08 (Ahrens 1994). It is generally recognized that plants can remediate organic pollutants by (1) direct root

uptake of contaminants and subsequent accumulation of nonphytotoxic metabolites in plant tissue, (2) direct foliar uptake of volatile contaminants from the surrounding air, and (3) release of exudates and enzymes that enhance biochemical transformations or mineralization due to mycorrhizal fungi and microbial activity in the rhizosphere (Schnoor et al. 1995). Anderson et al. (1993) and Anderson and Coats (1994) have reviewed bioremediation in the rhizosphere by microbial and biochemical processes.

Research reported in this paper was part of a larger project concerned with the development of a porous, root zone-based, constructed wetland for remediation of pesticide-contaminated rinsates. In the development of such a system, it was imperative that resident plant health not be compromised by excessive pesticide concentrations. Likewise, knowledge of uptake capacities and distribution within the plant was essential. Most work with plant uptake and distribution of herbicides reported in the literature has centered on ascertaining or enhancing herbicidal mode of action. In their literature review on triazine uptake, translocation, and degradation in plants, Esser et al. (1975) reported that root uptake readily occurred with all plants studied, regardless of whether they were resistant or susceptible to the herbicides. They also reported that increasing concentrations and time of exposure resulted in increased uptake. Uptake rates were also accelerated by high temperatures and low relative humidity (Esser et al. 1975). As a class, the triazines were seen to be evenly distributed through the xylem into all aerial parts of plants following root uptake. Specific plant species that have been shown to readily transport triazines acropetally from roots to leaves include: *Zea mays* L. (corn), *Gossypium hirsutum* L. (cotton), and *Cucumis sativus* L. (cucumber) (Davis et al. 1959), *Picea abies* L. (Norway spruce) (Lund-Hoie 1969), *Juglans nigra* L. (black walnut) and *Liriodendron tulipifera* L. (yellow poplar) (Wichman and Byrnes 1975), poplar clones (Akinyemiju et al. 1983), *Raphanus sativus* L. (radish) seedlings (Shone and Wood 1976), and *Hordeum vulgare* L. (barley) (Shone and Wood 1972, 1974; Shone et al. 1974). Very little work on simazine uptake has been reported in the context of phytoremediation, with the exception of Burael and Fuhr (1988), who reported enhanced mineralization of simazine in soil following uptake by *Z. mays* and breakdown of the plant.

Specific objectives of this research included (1) establishing threshold tolerances to simazine, (2) quantifying [¹⁴C]-simazine uptake into the plant from a spiked solution, and (3) determining the distribution of [¹⁴C]-simazine throughout the plant.

Materials and Methods

Plant Material

Acorus gramenius belongs to the Araceae Juss. family. These plants are aromatic with thick, creeping rhizomes and erect, linear ensiform leaves crowded at the base (Correll and Correll 1975). Several varieties of *A. gramenius* are sold as ornamentals for water gardening. This plant is a rooted, emergent species that grows up to 46 cm tall.

Pontederia cordata belongs to the Pontederiaceae H.B.K. family. These emergent wetland plants are stout, perennial herbs with thick, creeping rhizomes rooted in bottom sed-

iments (Correll and Correll 1975). They may grow as tall as 1 m. Leaves are erect and long-petioled, vary in shape from deltoid-ovate to triangular-lanceolate, and are prominently and deeply cordate to truncate at the base (Correll and Correll 1975). Ducks and muskrats have been reported to eat the seeds of *Pontederia* (Correll and Correll 1975). These plants are commonly found in marshes and along sluggish streams and ditches in shallow water. Many varieties of this species are sold as ornamentals.

Original plant stocks were obtained from Carolina Nursery¹ and Head-Lee Nursery.² Test plants were propagated from these stocks by division and planting in Fafard³ Germination Mix in a greenhouse. Plants were watered and fertilized (Peter's Lite Special⁴ water-soluble fertilizer, 20-10-20) as needed. Approximately 3 to 4 wk before tests were initiated, plants were transferred from potting media in the greenhouse to hydroponics in the lab. The hydroponic system consisted of several 2-L polypropylene containers filled with 10% Hoagland's nutrient solution. The nutrient solution was not sterilized before use and never became cloudy during plant culture or during the evaluations. Four to eight plants were grown in each container during the acclimation periods. The nutrient solution was changed weekly. Nutrient media lost due to evapotranspiration was replaced with distilled, deionized water.

Toxicity Assessment

Toxicity tests were conducted at 25 ± 2 C under metal halide lamps with a photon flux of 375 ± 25 μmol m⁻² s⁻¹ and a 16:8 h light : dark photoperiod. Technical-grade simazine (reported purity: 99.6%) was obtained from Novartis Crop Protection.⁵ Treatment solutions were made by dissolving simazine overnight in 10% Hoagland's nutrient media at the following concentrations: 0, 0.01, 0.03, 0.1, 0.3, 1.0, and 3.0 mg L⁻¹. These concentrations were chosen to bracket known effective concentrations reported in the literature using other plant species (Fowler 1977; O'Neal and Lembi 1983). Simazine concentrations pre-exposure were confirmed using SDI RaPID Imuno Assays.⁶ Previous experience showed comparable results between the assays and standard gas chromatographic analysis. Simazine concentrations were not measured at the end of the exposure period. Individual plants were exposed in glass jars to 250 ml of each pesticide concentration for 7 d. Plant roots were rinsed with distilled water after the 7-d exposure period. Plants were then placed in "clean" nutrient media and allowed to grow an additional 7 d to observe any latent effects or recovery. All exposure jars were autoclaved before treatment. Each jar was also covered with aluminum foil to exclude extraneous light from the root zone and exposure media. Each plant was held in place using the lower half of a 236-ml foam cup with a hole cut in the bottom.

Fresh weights were recorded before exposures, after 7-d exposure, and after the 7-d postexposure period. These measurements were used to calculate the fresh weight gains during the 7-d exposure and postexposure periods. Chlorophyll fluorescence parameters were also measured on intact *P. cordata* plants at the same time intervals using an OPTISCIENCES OS-500 modulated fluorometer.⁷ These measurements were not taken with *A. gramenius* because of the small width of their leaves. These measurements are typically used to examine the physiological status of the photosynthetic appa-

ratus and are based on the fact that 3 to 5% of the light energy absorbed by chlorophyll pigments is re-emitted as fluorescence under normal conditions (Karukstis 1991; Krause and Weis 1984; Miles 1990). Photosynthesis-inhibiting herbicides and other substances or environmental conditions that alter the overall bioenergetic status of the plant induce characteristic changes in fluorescence emissions (Hipkins and Baker 1986). The reader is referred to the above-referenced works and to those by Lower et al. (1990) and Krugh and Miles (1996) for a complete discussion of the principles and utility of chlorophyll fluorescence measurements in plant experiments.

Chlorophyll fluorescence emission parameters measured included initial fluorescence (F_0), maximal fluorescence (F_m), steady-state fluorescence (F_s), and maximal steady-state fluorescence (F_{ms}). Illumination following a dark period causes a rapid rise to F_0 (initial chlorophyll fluorescence level), which is a measurement of the fluorescence emission from chlorophyll antenna molecules before energy has migrated to the PSII reaction centers (Krugh and Miles 1996). Fluorescence from the PSII chlorophyll *a* antenna molecules increases to a maximum level (F_m) when the first stable electron acceptor (Q_A) is fully reduced (Krugh and Miles 1996). The variable fluorescence (F_v) represents the reduction of PSII electron acceptors and is calculated as $F_m - F_0$ (Krugh and Miles 1996). The ratio of F_v/F_m is a measure of the quantum efficiency of the plant. Plants were dark-adapted for 20 min before F_0 and F_m measurements were taken. The parameters F_s and F_{ms} refer to the fluorescence emissions of chlorophyll *a* antenna molecules in PSII under steady state, lighted conditions. F_s is comparable to F_0 , except that it is under steady-state conditions while photosynthesis is occurring, as opposed to the initial conditions, where all electron acceptors were fully oxidized. F_{ms} is the maximal fluorescence emission from PSII chlorophyll *a* molecules during exposure to a source of light-driving photosynthesis (Anonymous 1995b). The steady-state fluorescence emission yield is a measure of quantum efficiency during photosynthesis and is calculated as $(F_{ms} - F_s)/F_{ms}$.

A completely randomized statistical design with four replications for each exposure concentration was used. Fresh-weight gain data were ranked and analyzed by analysis of variance (ANOVA) at $P = 0.05$. Results were further analyzed using calculated LSD. Fluorescence data were analyzed by ANOVA at $P = 0.05$.

Uptake and Distribution

The uptake and distribution of simazine by *A. gramineus* and *P. cordata* was evaluated in different experiments using [^{14}C]-simazine labeled at the two, four, and six positions in the triazine ring. This material was obtained from Novartis Crop Protection⁵ and had a reported chemical purity of 98.7% and radio-purity of 96%. The specific activity of the [^{14}C]-simazine was 1.12×10^6 Bq mg^{-1} . Approximately 1.04×10^6 Bq of [^{14}C]-simazine were dissolved overnight in 3.8 L of 10% Hoagland's nutrient media. This was equivalent to a concentration of approximately 0.24 mg L^{-1} . For each exposure, 14- to 250-ml sidearm vacuum flasks were each filled with 275 ml of the spiked nutrient media. Non-spiked nutrient media served as untreated controls and were treated similarly. The side arms of the exposure flasks were equipped with one-way valves that allowed movement of

gases from the outside of the flask to the inside. Individual plants were held in place (with roots submerged in the spiked or nonspiked nutrient media) by #6 silicon stoppers with holes bored through the tops and a slit along the side. Stoppers were wrapped around individual plant stems and sealed using Qubitac⁸ nontoxic, nonreactive putty. A smaller hole in the stopper was fitted with a teflon tube (1.6 mm i.d., 3.1 mm o.d.) that was connected to an in-line [^{14}C]- CO_2 and $\text{VO}^{[14\text{C}]}$ trap. The traps contained 15 ml of 0.5 M NaOH and 2 g of 20- to 60-mesh activated charcoal,⁹ respectively. Two flasks containing the spiked nutrient media, but without plants, were also included as references. These reference flasks were equipped and handled in the same manner as the others.

Once plants were secured in the exposure vessels, they were transferred to a Conviron¹⁰ CMP3244 environmentally controlled growth chamber (photon flux, $375 \pm 25 \mu\text{mol m}^{-2} \text{ s}^{-1}$ generated by fluorescent and incandescent lamps; photoperiod, 16:8 h light : dark; relative humidity 60%; temperature, 25:22 C light : dark). Headspace within each flask was purged through the scrubbers daily with two to three volumes of air using a 60-ml syringe attached to the side-arm one-way valve assembly. Water transpired through the plant was replenished with distilled, deionized water using the same syringe. Water use was recorded daily.

On days 1, 3, 5, and 7, three exposed and two control plants were randomly harvested. Plant rhizomes and roots were rinsed in running tap water for 45 s and were blotted dry. Plants were then dissected into individual leaves, stems, rhizomes, and roots. Fresh weights for each individual section were recorded. Individual sections were wrapped in aluminum foil, flash frozen using liquid nitrogen, and stored at -80 C until the tissues could be analyzed. At the time of analysis, plant tissues were freeze dried, weighed, and combusted using an R. J. Harvey¹¹ biological oxidizer at 900 C for 3 min. [^{14}C]- CO_2 generated by combustion was captured using an R. J. Harvey¹¹ [^{14}C]- CO_2 trapping cocktail. The captured [^{14}C]- CO_2 content was analyzed using a Beckman¹² LS 6500 liquid scintillation counter. Each sample was counted for 8 min in the DPM (disintegrations per minute) mode.

Exposure Solution Analysis

In addition to harvesting the plant tissue, samples of the exposure solutions were analyzed for total [^{14}C] content. Total [^{14}C] content was assessed by adding 0.2 ml of the exposure solution to 6 ml Scinti Verse BD¹³ scintillation cocktail. These samples were then counted for 8 min using a Beckman¹² LS 6500 liquid scintillation counter in the DPM mode.

Disposition of simazine in the exposure solution was determined by high-pressure liquid chromatography (HPLC). Samples (3 ml) were filtered through 0.2- μm PTFE Acrodiscs.¹⁴ The filtered samples were analyzed using a Waters¹⁵ 600E HPLC system controller equipped with a Waters Nova-Pak¹⁵ C₁₈ radial compression analytical column (4- μm particle size, 60- \AA pore size, 8 by 100 mm), β -RAM¹⁶ yttrium silicate radiochemical detector and a Waters¹⁵ 484 tunable ultraviolet (UV) absorbance detector (λ : 254 nm). The mobile phase consisted of a gradient of 10:90% to 70:30% MeOH : H_2O in 30 min. The injection volume was 0.700 ml and the flow rate was adjusted to 1.5 ml min^{-1} .

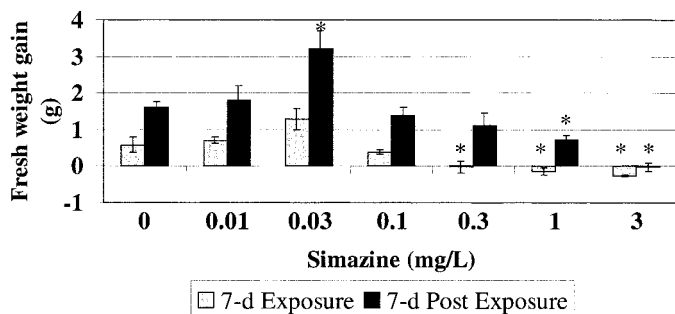


FIGURE 1. Effects of simazine on fresh weight gains of *Acorus gramenius* after a 7-d exposure and 7-d postexposure period. Bars represent standard errors of the mean. Statistical comparisons are only valid within exposure categories. An asterisk (*) indicates significant difference from controls ($P = 0.05$).

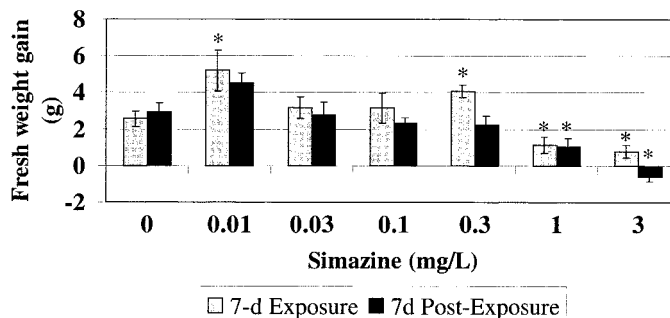


FIGURE 2. Effects of simazine on fresh weight gains of *Pontederia cordata* after a 7-d exposure and 7-d postexposure period. Bars represent standard errors of the mean. Statistical comparisons are only valid within exposure categories. An asterisk (*) indicates significant difference from controls ($P = 0.05$).

Results and Discussion

Toxicity

A. gramenius

Fresh weight gains for *A. gramenius* exposed to simazine were significantly reduced at the 0.3, 1, and 3 mg L⁻¹ treatment levels after the 7-d exposure period (Figure 1). These reductions amounted to 103, 124, and 144% of the controls, respectively. Following the 7-d postexposure period, plants exposed to the 0.3 and 1 mg L⁻¹ treatment concentrations displayed an exposure-dependent recovery inversely related to concentration. Fresh weight gains during this period increased to 71 and 46%, respectively, relative to the controls. Fresh weight gains for plants exposed to 0.3 mg L⁻¹ were comparable to the controls during the 7-d postexposure period. However, fresh weight gains for plants exposed to 1 mg L⁻¹ simazine remained statistically lower than the controls. Fresh weight gains for plants exposed to 3 mg L⁻¹ simazine were -1% relative to the controls. This negative increase in fresh weight was due to the desiccation of plant tissue associated with senescence. Affected plants displayed symptoms of tip necrosis and chlorosis after exposures of 5 to 7 d. Roots of unaffected plants were brightly colored whitish-tan and massive compared to those of affected plants. Roots of affected plants were darkly colored, less turgid, and lacked extensive new growth.

P. cordata

Fresh weight production for plants exposed to simazine was reduced at the higher treatment levels after the 7-d exposure and 7-d postexposure periods (Figure 2). Seven-day fresh weight gains for plants exposed to 0.01, 0.03, 0.1, and 0.3 mg L⁻¹ were equal to or greater than those of the controls (Figure 2). However, fresh weight gains for those exposed to 1.0 and 3.0 mg L⁻¹ were only 34 and 30% of the controls, respectively. Plants exposed to 3 mg L⁻¹ senesced by the end of the 7-d postexposure period. No latent effects were observed for plants exposed to 0.01, 0.03, 0.1, and 0.3 mg L⁻¹ during the 7-d postexposure period. However, fresh weight gains were only 35 and -20% of the controls for plants exposed to 1 and 3 mg L⁻¹ simazine, respectively, during the 7-d postexposure period. Chlorosis of affected plants was visible after approximately 6 d exposure, with the appearance of marginal necrotic lesions occurring shortly thereafter.

Chlorophyll fluorescence measurements with dark-adapted leaves showed an exposure-dependent reduction in F_v/F_m ratios by day 1 (Table 1). These reductions were significantly different from the controls at 0.3, 1, and 3 mg L⁻¹ simazine from day 1 through day 7 of exposure. F_v/F_m ratios for plants exposed to the 0.1 mg L⁻¹ treatment level were significantly lower than the controls after 5 d exposure but recovered afterward. F_v/F_m ratios were 80, 54, and 28% of the controls for the 0.3, 1.0, and 3.0 mg L⁻¹ treatment levels after 7 d exposure, respectively. F_v/F_m ratios for plants exposed to 0.3 and 1 mg L⁻¹ simazine recovered to the control levels after transfer to clean media, indicating reversible photoinhibition. However, F_v/F_m ratios for plants exposed to 3 mg L⁻¹ simazine remained lower than for the controls. The decrease in F_v/F_m ratios at higher simazine concentrations resulted from increased F_0 (initial fluorescence) associated with the blockage of the electron transport chain after Q_A . F_0 was more than twice that of the controls for plants exposed to 1 and 3 mg L⁻¹ simazine for 7 d.

An exposure-dependent reduction in quantum efficiency as measured by the light-adapted fluorescence yield [$(F_{ms} - F_s)/F_{ms}$] was also apparent (Table 2). Fluorescence yields for plants exposed to the 0.01 mg L⁻¹ treatment concentration were statistically similar to the controls throughout the 14-d experimental period. Fluorescence yields for plants exposed to the 0.3, 1.0, and 3.0 mg L⁻¹ concentrations were significantly reduced after 1 d. Fluorescence yields were significantly reduced for the 0.03, 0.1, 0.3, 1, and 3 mg L⁻¹ treatments from 3 to 7 d. Yields for plants exposed to 0.01 and 0.03 mg L⁻¹ simazine were similar to the controls after the 7-d postexposure period, whereas all the rest were reduced. These reductions in quantum efficiencies were due to increases in F_s (normal steady-state fluorescence during active photosynthesis) (Table 2).

The no observable effects concentration (NOEC)/lowest observable effects concentration (LOEC) ranges found for both species are similar to those reported for several aquatic micro- and macrophytes. Photosynthesis was inhibited 50% in filamentous algae at 0.22 to 0.95 mg L⁻¹ and at 0.95 mg L⁻¹ simazine in nonfilamentous algae (O'Neal and Lembi 1983). Likewise, *M. verticillatum*, *P. pectinatus*, and *V. dichotoma* senesced at simazine concentrations of 1 mg L⁻¹, whereas *R. heiroglypticum* and *C. glomerata* senesced at 0.5 mg L⁻¹ (Fowler 1977). However, toxicity thresholds for our study were much higher than those reported for *Myriophyllum spicatum* (Salah et al. 1989). These researchers devel-

TABLE 1. Initial fluorescence (F_0), maximal fluorescence (F_m), and quantum efficiency ($F_v/F_m = [F_m - F_0]/F_m$) values for dark-adapted *Pontederia cordata* exposed to simazine for 7 d and after a 7-d postexposure period.^a F_0 , F_m , and F_v/F_m are unitless.

Simazine	Day	F_0 (SD)	F_m (SD)	F_v/F_m (SD)
mg L ⁻¹				
0	0	291 (18.6)	1,766.7 (10.7)	0.834 (0.012)
	1	287.5 (14.1)	1,644 (93.2)	0.824 (0.017)
	3	284.7 (24.5)	1,613.2 (41.1)	0.823 (0.015)
	5	302 (25.8)	1,729.5 (48.1)	0.824 (0.018)
	7	304 (32.3)	1,694.7 (87.5)	0.82 (0.02)
	14	280 (11.5)	1,583 (61.1)	0.822 (0.009)
0.01	0	308.7 (16.9)	1,808.2 (100.5)	0.828 (0.013)
	1	279.2 (9.3)	1,649.5 (95.1)	0.829 (0.015)
	3	292.5 (14.9)	1,642.5 (134.0)	0.820 (0.019)
	5	312 (28.0)	1,713.5 (37.9)	0.817 (0.019)
	7	300.2 (26.1)	1,614.2 (125.8)	0.813 (0.02)
	14	280.7 (27.1)	1,588.2 (50.4)	0.822 (0.018)
0.03	0	272.75 (31.8)	1,693.5 (112.5)	0.838 (0.014)
	1	275.7 (13.6)	1,574.0 (74.0)	0.824 (0.014)
	3	285.5 (17.9)	1,617.2 (46.7)	0.823 (0.007)
	5	327.7 (14.5)	1,770.5 (52.0)	0.814 (0.009)
	7	319.7 (12.7)	1,709 (119.2)	0.811 (0.017)
	14	312.2 (20.8)	1,528.5 (123.1)	0.793 (0.03)
0.1	0	307.5 (8.3)	1,846.5 (101.6)	0.832 (0.010)
	1	284.7 (6.6)	1,708.0 (98.6)	0.832 (0.007)
	3	351.0 (62.9)	1,669.7 (95.0)	0.787 (0.047)
	5	411 (36.9)	1,833.7 (123.3)	0.774 (0.025)*
	7	386.2 (40.0)	1,851.5 (175.1)	0.79 (0.023)
	14	308.7 (18.5)	1,680 (71.3)	0.815 (0.005)
0.3	0	289.7 (12.1)	1,778.7 (57.1)	0.836 (0.008)
	1	409.7 (71.4)	1,526.5 (128.2)	0.727 (0.068)*
	3	510 (74.0)	1,567.2 (144.1)	0.669 (0.074)*
	5	678.7 (87.1)	1,810.2 (144.1)	0.625 (0.029)*
	7	571.5 (101.6)	1,667.2 (171.5)	0.651 (0.095)*
	14	310.2 (11.7)	1,693.7 (105.9)	0.815 (0.016)
1.0	0	299.2 (11.5)	1,839.0 (85.3)	0.836 (0.005)
	1	487.5 (95.3)	1,510 (210.5)	0.667 (0.092)*
	3	613.7 (56.5)	1,558.7 (98.1)	0.604 (0.051)*
	5	789.2 (112.1)	1,585.7 (395.4)	0.472 (0.177)*
	7	783.2 (56.8)	1,452.5 (350.1)	0.44 (0.128)*
	14	275 (24.9)	1,706.2 (68.0)	0.838 (0.019)
3.0	0	321.0 (11.5)	1,861.2 (30.9)	0.827 (0.006)
	1	563.0 (21.4)	1,435.5 (133.0)	0.605 (0.034)*
	3	709.7 (49.7)	1,722 (261.1)	0.583 (0.038)*
	5	993.7 (152.8)	1,587.2 (221.0)	0.363 (0.131)*
	7	884.2 (74.8)	1,227 (326.5)	0.242 (0.184)*
	14	427 (36.1)	1,786.3 (156.1)	0.758 (0.038)*

^a Significantly different from controls at * $P = 0.05$.

oped a bioassay for photosynthetic inhibitors in water and aqueous soil extracts using *M. spicatum*. Based on their results, oxygen generation was reduced 50% at 20 $\mu\text{g L}^{-1}$ simazine in water.

Based on these results, some caution is warranted when using plants for the phytoremediation of simazine. However, one must realize that these tests represented a worst-case scenario, in which other substrates capable of reducing bioavailability were not present. Under natural conditions in the field, significant portions of each pesticide may be sorbed to substrates or degraded by microorganisms and sunlight. In the case of a gravel-based phytoremediation system designed for removing simazine from water, caution is still warranted due to the expected pesticide concentrations. Expected simazine concentrations in 568 L of rinse water were calculated to range from 9.2 to 92.5 mg L^{-1} , assuming a moderate

application rate, 378 L original mix volume, and a residual rinse volume ranging from 3.8 to 37.9 L. These pesticide concentrations far exceed the tolerance thresholds for simazine. Because the toxicity of the compounds to the individual plant species cannot be changed, a possible strategy for reducing risks to the resident plant species may be through dilution with "clean" water. Field studies characterizing the bioavailable fraction are needed to better evaluate risks.

Uptake and Distribution

A. gramenius

[¹⁴C]-simazine activity in the exposure solutions decreased with time (Table 3). These reductions were 20, 28, 36, and 45%, respectively, after 1, 3, 5, or 7 d of exposure.

TABLE 2. Steady-state fluorescence (F_s), maximal fluorescence (F_{ms}), and quantum efficiency measured as fluorescence yield ($Y = (F_{ms} - F_s)/F_{ms}$) values for actively photosynthesizing *Pontederia cordata* exposed to simazine for 7 d and after a 7-d postexposure period. F_s , F_{ms} , and Y are unitless.

Simazine	Day	F_s (SD)	F_{ms} (SD)	Y (SD)
mg L ⁻¹				
0	0	221 (47.7)	916.2 (93.9)	0.759 (0.037)
	1	260 (18.1)	1,003.5 (49.7)	0.74 (0.023)
	3	262.5 (18.6)	976.2 (43.8)	0.730 (0.021)
	5	247.2 (14.1)	953.2 (61.1)	0.738 (0.029)
	7	250.7 (13.5)	933.5 (19.7)	0.731 (0.02)
	14	209.7 (24.4)	950.7 (123.4)	0.778 (0.01)
0.01	0	247.5 (29.8)	937.2 (30.9)	0.735 (0.028)
	1	263.7 (31.1)	1,008 (32.4)	0.737 (0.036)
	3	272 (9.6)	1,083.7 (91.8)	0.747 (0.018)
	5	273.7 (20.9)	1,024 (98.0)	0.731 (0.016)
	7	242.7 (34.3)	917.2 (77.8)	0.735 (0.016)
	14	239.2 (15.13)	926.5 (190.83)	0.733 (0.054)
0.03	0	214.2 (6.1)	834 (80.1)	0.741 (0.023)
	1	254.7 (20.7)	954.5 (32.4)	0.732 (0.022)
	3	311 (26.1)	1,005.5 (10.9)	0.69 (0.028)*
	5	349.2 (32.3)	1,019.2 (27.8)	0.657 (0.025)*
	7	316 (42.4)	950 (64.6)	0.667 (0.03)*
	14	230 (32.2)	900 (83.5)	0.744 (0.013)
0.1	0	223 (6.3)	939.2 (52.5)	0.761 (0.018)
	1	281.7 (25.6)	1,082.2 (88.8)	0.738 (0.019)
	3	462 (185.0)	1,057 (60.5)	0.563 (0.168)*
	5	506 (66.5)	1,130.7 (106.2)	0.552 (0.043)*
	7	453.5 (88.3)	1,014.5 (66.9)	0.553 (0.07)*
	14	248.7 (19.7)	908 (30.3)	0.725 (0.02)*
0.3	0	222 (19.7)	929.5 (32.1)	0.761 (0.021)
	1	525 (171.4)	981 (94)	0.451 (0.209)*
	3	879.2 (101.32)	1,168.7 (163.6)	0.244 (0.038)*
	5	903.7 (21.7)	1,091.5 (20.5)	0.171 (0.026)*
	7	693 (60.7)	993.2 (45.9)	0.299 (0.086)*
	14	275.7 (32.9)	989.2 (70.2)	0.719 (0.044)*
1.0	0	221.7 (14.7)	942.2 (48.3)	0.763 (0.017)
	1	765 (180.8)	1,082.5 (104.2)	0.284 (0.185)*
	3	1,034.2 (97.5)	1,249.2 (165.5)	0.167 (0.049)*
	5	911.5 (143.3)	1,001.7 (200.8)	0.082 (0.051)*
	7	748.5 (148.7)	840.2 (211.6)	0.096 (0.088)*
	14	294.7 (38.2)	922.5 (72.0)	0.678 (0.053)*
3.0	0	245.75 (12.5)	922.2 (30.8)	0.733 (0.012)
	1	835 (61.2)	1,010 (97.3)	0.171 (0.025)*
	3	1,022 (152.8)	1,187.7 (151.1)	0.141 (0.031)*
	5	944 (105.6)	978.5 (136.6)	0.032 (0.029)*
	7	628.7 (195.0)	657.2 (234.3)	0.032 (0.04)*
	14	614.3 (32.1)	1,041.3 (19.0)	0.409 (0.039)*

^a Significantly different from controls at * $P = 0.05$.

Regression analysis indicated that the amount of activity remaining in the exposure solution was inversely proportional to the cumulative water used by each plant ($P = 1.99 \times 10^{-7}$; % remaining = $-0.00216[\text{cumulative water used (ml)}] + 0.8568$, $R^2 = 0.94$). A radio impurity/metabolite was detected in the exposure solutions using HPLC. The increase in activity due to an unidentified impurity/metabolite toward the end of the exposure period may have resulted from simazine breakdown in plant-containing flasks. Nevertheless, because impurity/metabolite levels in these flasks remained relatively constant, the assumption that the activity detected in plants was due primarily to uptake of parent simazine was valid. Simazine levels within reference flasks containing no plants remained relatively constant throughout the experimental period (Table 3). Likewise, im-

purity levels within the reference flasks remained relatively constant (2 to 7% of total activity) with very low coefficients of variation (Table 3).

Nearly all of the [¹⁴C] activity removed from the exposure solution was detected in the plants. After 1, 3, 5, and 7 d exposure, 6.6, 15.7, 21.2, and 29.8% of the total activity added, respectively, was detected in the whole plants. The mass balance for [¹⁴C] was consistent throughout the test, with 13 to 15% of the original activity being unmeasured at the end of the exposure period (Table 3). Some of the activity not accounted for may have been lost when plant roots were rinsed under the tap. Rinse water was not analyzed for radioactivity. Also, the headspace within each exposure vessel was not analyzed for [¹⁴C]-CO₂ or volatilized compounds due to difficulties in obtaining an airtight

TABLE 3. Percent^a distribution of total [¹⁴C] activity initially present in exposure solutions with or without *Acorus gramenius*.

Fraction ^b	Day 1		Day 3		Day 5		Day 7	
	<i>n</i> = 3	CV ^c	<i>n</i> = 3	CV	<i>n</i> = 3	CV	<i>n</i> = 3	CV
Experimental vessels with <i>A. gramenius</i>								
Solution	0.78	<i>0.01</i>	0.64	<i>0.08</i>	0.54	<i>0.05</i>	0.46	<i>0.05</i>
Impurity	0.02	<i>0.01</i>	0.08	<i>0.08</i>	0.10	<i>0.05</i>	0.09	<i>0.05</i>
Roots	0.028	<i>0.159</i>	0.047	<i>0.237</i>	0.052	<i>0.092</i>	0.067	<i>0.138</i>
Rhizomes	0.009	<i>0.223</i>	0.023	<i>0.472</i>	0.016	<i>0.177</i>	0.022	<i>0.148</i>
Leaves	0.028	<i>0.336</i>	0.086	<i>0.32</i>	0.144	<i>0.094</i>	0.209	<i>0.115</i>
Unaccounted	0.135	—	0.124	—	0.148	—	0.152	—
Reference vessels without <i>A. gramenius</i>								
Solution	0.85	<i>0.01</i>	0.81	<i>0.01</i>	0.82	<i>0.01</i>	0.82	<i>0.02</i>
Impurity	0.02	<i>0.01</i>	0.07	<i>0.01</i>	0.06	<i>0.01</i>	0.06	<i>0.02</i>

^a Fractional form.^b Solution, simazine in exposure solution; impurity, unidentified impurity and/or metabolite in exposure solution.^c CV, coefficient of variation. Italics indicate % detected.

seal between the rhizosphere and the outside of the vessel. It is possible that some of the unmeasured activity left the flask as [¹⁴C]-CO₂ or volatilized simazine. However, losses due to volatilization are not expected to be large given simazine's Henry's Law constant of 9.48×10^{-10} atm-m³ mol⁻¹ (Ahrens 1994). Regression analyses indicated that accumulation of activity in the whole plant was directly proportional to cumulative water use by the plant ($P = 7.55 \times 10^{-7}$; % accumulated = $0.001914[\text{cumulative water used (ml)}] + 0.02895$, $R^2 = 0.92$).

Analysis of the dissected plant tissues revealed significant accumulations of [¹⁴C] activity, primarily in the leaves and to a lesser extent in the roots (Table 3). No accumulation was seen in the rhizomes, indicating that they serve primarily as a pathway for acropetal transport.

P. cordata

[¹⁴C]-simazine activity in the exposure solutions decreased with time (Table 4). These reductions were 6, 25, 22, and 34% after 1, 3, 5, and 7 d exposure, respectively. Regression analysis revealed that the amount of activity remaining in the exposure solution was inversely proportional to the cumulative amount of water used by the plants ($P =$

1.19×10^{-8} ; % remaining = $-0.00189[\text{cumulative water used (ml)}] + 0.98219$, $R^2 = 0.97$). No significant amount of [¹⁴C] was detected in the NaOH scrubbers, indicating little loss due to mineralization in the rhizosphere. Though mineralization of [¹⁴C]-simazine by the plant shoots was not measured, Shone and Wood (1972) found no [¹⁴C]-CO₂ in air passed over barley plants that were dosed with 0.200 mg L⁻¹ for 48 h. Losses due to volatilization were probably not significant because of simazine's low vapor pressure of 1.5×10^{-8} mm Hg and Henry's Law constant of 9.5×10^{-10} atm-m³ mol⁻¹ (Ahrens 1994). The radio impurity/metabolite detected by HPLC remained relatively constant throughout the experimental period. This impurity was not identified but appeared to be more polar than simazine, as evidenced by its shorter retention time of 12 min compared to simazine's at 20 min. Because impurity/metabolite levels in these flasks remained relatively constant, the assumption that the activity detected in plants was due primarily to uptake of parent simazine was valid.

Nearly all of the [¹⁴C]-simazine removed from the exposure solution was detected in the plants. After 1, 3, 5, and 7 d exposure, 7, 20, 24, and 32% of the total activity added, respectively, was detected in whole plants. The mass balance for [¹⁴C] was good throughout the test, with un-

TABLE 4. Percent^a distribution of total [¹⁴C] activity initially present in exposure solutions with or without *Pontederia cordata*.

Fraction ^b	Day 1		Day 3		Day 5		Day 7	
	<i>n</i> = 3	CV ^c	<i>n</i> = 3	CV	<i>n</i> = 3	CV	<i>n</i> = 3	CV
Experimental vessels with <i>P. cordata</i>								
Solution	0.88	<i>0.02</i>	0.75	<i>0.07</i>	0.72	<i>0.07</i>	0.61	<i>0.20</i>
Impurity	0.07	<i>0.02</i>	0.06	<i>0.06</i>	0.06	<i>0.07</i>	0.05	<i>0.20</i>
Roots	0.015	<i>0.527</i>	0.033	<i>0.427</i>	0.045	<i>0.511</i>	0.036	<i>0.220</i>
Rhizomes	0.027	<i>0.476</i>	0.055	<i>0.138</i>	0.043	<i>0.389</i>	0.066	<i>0.342</i>
Leaf petioles	0.017	<i>0.520</i>	0.049	<i>0.216</i>	0.052	<i>0.164</i>	0.089	<i>0.739</i>
Leaf blades	0.005	<i>0.757</i>	0.063	<i>0.613</i>	0.102	<i>0.177</i>	0.147	<i>0.447</i>
Unaccounted	0.014	—	+0.01	—	+0.022	—	0.002	—
Reference vessels without <i>P. cordata</i>								
Solution	0.93	<i>0.01</i>	0.95	<i>0.00</i>	0.96	<i>0.003</i>	0.98	<i>0.004</i>
Impurity	0.08	<i>0.01</i>	0.08	<i>0.00</i>	0.08	<i>0.003</i>	0.08	<i>0.004</i>

^a Fractional form.^b Solution, simazine in exposure solution; impurity, unidentified impurity and/or metabolite in exposure solution; +, activity in excess of what was originally added.^c CV, coefficient of variation. Italics indicate % detected.

measured label never exceeding 5% (Table 4). Some of the activity not accounted for throughout the experimental period may have been lost when plant roots were rinsed under the tap. Rinse water was not analyzed for activity. Regression analyses revealed that the amount of activity accumulated within plants was directly proportional to cumulative water use by the plants ($P = 9.01 \times 10^{-5}$; % accumulated = $0.001533[\text{cumulative water used (ml)}] + 0.061331$, $R^2 = 0.80$).

Analysis of the dissected plant tissues revealed accumulation of [^{14}C] in all plant parts after 3 d exposure (Table 4). However, the majority of the label accumulated in the leaf petioles and blades after 7 d exposure.

Although these results indicate that these ornamental plant species may be useful for removing simazine from contaminated water, it is important to realize that in these scenarios, all of the pesticides were in solution, and uptake was through the roots. In order for these plants to be used effectively in the field, arrangements must be made to maximize root contact with the water column contaminants (i.e., porous substrate, etc.). Additionally, under most field conditions, sorption to soils and organic materials would reduce the bioavailability of simazine, limiting potential uptake of the chemical. Scribner et al. (1992) reported that aged simazine residues were unavailable for *Beta vulgaris* L. (sugar beet) uptake and to microbial degraders, as opposed to recently added simazine. Jones and Estes (1984) reported reduced bioavailability of atrazine to *Potamogeton perfoliatus* L. due to soil sorption.

One question that this research did not approach is the fate of simazine once it is in the plants. This was not possible because of the destructive nature of combusting the samples. However, it is likely that some metabolism occurred. Burauel and Fuhr (1988) suggested that plant metabolism was responsible for the greater degree of simazine mineralization in their soil following uptake by *Z. mays* and desiccation of the plant. They suggested that the simazine was more readily degradable by microorganisms following plant metabolism. Likely metabolic products include hydroxy-simazine, mono- and di-dealkylated simazine, and various conjugates (Akinyemiju et al. 1983; Beyton et al. 1972; Burnet et al. 1993; Castelfranco et al. 1961; Esser et al. 1975; Funderburk and Davis 1963). However, another study found no evidence of extensive simazine breakdown in xylem sap of *H. vulgare* (barley) (Shone and Wood 1972).

Phytotoxicity results indicated that highest NOECs and the LOECs for both species were 0.3 and 1 mg L⁻¹ using fresh weight gains after the 7-d postexposure period as the measured endpoint. Senescence always occurred at concentrations of 3 mg L⁻¹. Approximately 45 and 34% of the herbicide initially present in the water was removed by *A. gramineus* and *P. cordata*, respectively, within 7 d. Plant-incorporated simazine was acropetally distributed from the roots primarily into the leaves. Simazine uptake was directly related to water uptake with the transpiration stream.

Future work will evaluate the actual phytoremedial ability of these plants in constructed wetlands at The Walker Course, Clemson, South Carolina. Other ornamental plant species will also be evaluated for their phytoremedial abilities.

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Sources of Materials

- ¹ Carolina Nurseries Inc., 739 Gaillard Road, Monks Corner, SC 29461-5201.
- ² Head-Lee Nursery, Walhalla Highway, Walhalla, SC 29691.
- ³ Fafard Inc., 1471 Amity Road, Anderson, SC 29621.
- ⁴ Scotts Company, Huntington Center, Columbus, OH 43207.
- ⁵ Novartis Crop Protection, P.O. Box 18300, 410 Swing Road, Greensboro, NC 27419-8300.
- ⁶ Strategic Diagnostics Inc., 111 Pencader Drive, Newark, DE 19702-3322.
- ⁷ PP Systems, 241 Winter Street, Haverhill, MA 01830.
- ⁸ Qubit Systems Inc., 134 Albert Street, Kingston, ON, Canada K7L 3V2.
- ⁹ Sigma, St. Louis, MO.
- ¹⁰ Controlled Environments Limited, 590 Berry Street, Winnipeg, MB, Canada R3H 0R9.
- ¹¹ R.J. Harvey Instrument Corp., 123 Patterson Street, Hillsdale, NJ 07642.
- ¹² Beckman Inc., 4300 N. Harbor Boulevard, P.O. Box 3100, Fullerton, CA 92834-3100.
- ¹³ Fisher Scientific, 585 Alpha Dr., Pittsburgh, PA 15238.
- ¹⁴ Gelman Sciences Inc., 600 South Wagner Road, Ann Arbor, MI 48106.
- ¹⁵ Millipore Corporation, 80 Ashby Road, Bedford, MA 01730-2271.
- ¹⁶ IN/US Systems Inc., 5809 North 50th Street, Tampa, FL 33610-4809.

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