ABSTRACT

Phytoremediation depends on the ability of plants to tolerate and assimilate contaminants. This research characterized the interaction between several ornamental plant species and the fungicidal active ingredient, metalaxyl [N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine methyl ester]. Species evaluated included sweetflag (Acorus gramineus Sol. ex Aiton), canna (Canna hybrida L. \textit{‘Yellow King Humbert’}), parrotfeather \textit{(Myriophyllum aquaticum} (Vell.) Verdc.), and pickerelweed \textit{(Pontederia cordata} L.). Metalaxyl tolerance levels for each species were determined by exposing plants for 7 d to solutions containing 0, 5, 10, 25, 50, 75, or 100 mg metalaxyl L$^{-1}$ aqueous nutrient media. Response endpoints included fresh mass production after 7 d exposure and 7 d post-exposure and quantum efficiency using dark-adapted (Fv/Fm) and light-adapted (fluorescence yields) plants. Metalaxyl uptake and distribution within the plant was determined by growing plants in aqueous nutrient media containing 1.18 $\times$ 10$^4$ Bq L$^{-1}$ [$^{14}$C]metalaxyl (0.909 mg L$^{-1}$) for 1, 3, 5, or 7 d. Plant tissues were combusted and analyzed by liquid scintillation counting. Metalaxyl had no effects on the endpoints measured, except for fresh mass production of sweetflag at the 75 and 100 mg L$^{-1}$ treatment levels. However, leaf necrosis was apparent in most species after 5 d exposure to concentrations greater than 25 mg L$^{-1}$. Metalaxyl removal from the spiked nutrient media ranged from 15 to 60% during the 7-d exposure period. The majority of metalaxyl removed from the solution was detected within individual plants. In nearly all cases, activity from the radiolabeled pesticide accumulated in the leaves. Uptake of metalaxyl was correlated with water uptake throughout the 7 d. These results suggest that all species examined may be good candidates for incorporation into a phytoremediation scheme for metalaxyl.

The turfgrass and nursery industries have become increasingly popular as specializations of production agriculture. These industries may generate significant amounts of pesticide-contaminated water by the common practice of rinsing application equipment after use. Most manufacturers recommend that the pesticide-containing rinsates be applied to areas that are normally treated. Improper disposal of these contaminated rinsates may significantly alter nontarget ecosystems. Phytoremediation offers one possible method for removing pesticides 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 and/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 depth), moderately hydrophobic pollutants (log $K_{ow}$ = 0.5–3), short-chain aliphatic chemicals, and excess nutrients. It is generally recognized that plants can remediate organic pollutants by (i) direct root uptake of contaminants and subsequent accumulation of nonphytotoxic metabolites in plant tissue, (ii) direct foliar uptake of volatile contaminants from the surrounding air by foliage, and (iii) release of exudates and enzymes that enhance biochemical transformations and/or mineralization due to mycorrhizal fungi and microbial activity in the rhizosphere (Schnoor et al., 1995; Anderson et al., 1993; Anderson and Coats, 1994).

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.

Metalaxyl was chosen as a model compound because of its common use by the turfgrass and ornamental horticulture industries, and because of its physicochemical properties. It is the fungicidal active ingredient of commercial formulations of Subdue (Novartis Crop Protection, Greensboro, NC). This systemic fungicide is very soluble in water (8.4 g L$^{-1}$ at 22°C) and is not volatile (vapor pressure: 5.62 $\times$ 10$^{-8}$ mm Hg at 25°C) (Wachope et al., 1992). The log $K_{ow}$ for this compound is 1.64 (Cohen and Coffey, 1986). This fungicide is available in granular, emulsifiable concentrate, and water-soluble-pouch commercial formulations. Subdue formulations are labeled for use in turfgrass and ornamentals production and landscaping. This fungicide effectively controls damping-off and root and stem diseases caused by \textit{Pythium} and \textit{Phytophthora} spp. Metalaxyl exists in two enantiomeric forms, each with significantly different biological activity. The R$^-$ enantiomer has significantly more antifungal activity than the S$^+$ enantiomer (Cohen and Coffey, 1986). Novartis has developed a technique for producing only the more biologically active enantiomeric R$^-$ form. These formulations are now marketed under the name Ridomil. However, it is anticipated that the older Subdue formulations will become generic of Florida-IFAS, 2199 South Rock Road, Fort Pierce, FL 34945-3138.

MATERIALS AND METHODS

Plant Material

The plant species evaluated in these studies were selected based on their aesthetic characteristics and hardiness to the southeastern USA. The plant species included sweetflag, canna, parrotfeather, and pickerelweed.

Original plant stocks were obtained from Carolina Nurseries (Monck’s Corner, SC) or Head-Lee Nursery (Seneca, SC). Canna were propagated from these stocks by taking 2.5- to 5-cm tuber cuttings and planting in Fafard (Anderson, SC) Germination Mix in a greenhouse. Sweetflag and pickerelweed were propagated by plant divisions and planted in the same germination mix. Parrotfeather was propagated by stem cuttings rooted in 10% Hoagland’s nutrient media (Hoagland and Arnon, 1938). Plants were watered and fertilized as needed using Scotts (Columbus, OH) Pete Light Special 20-10-20 water-soluble fertilizer. 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 1- to 2-L glass or polypropylene containers filled with 10% Hoagland’s nutrient solution. The nutrient solution was changed weekly. Liquid 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 density of 375 ± 25 μmol m⁻² s⁻¹ and a 16 h light–8 h dark photoperiod. Technical grade metalaxyl (reported purity: 95.8%) was obtained from Novartis Crop Protection. Treatment solutions were made by dissolving metalaxyl overnight in 10% Hoagland’s nutrient media at concentrations of 0, 5, 10, 25, 50, 75, and 100 mg L⁻¹. Metalaxyl concentrations were confirmed using SDI RaPID Immuno Assays (Strategic Diagnostics, Newark, DE) and gas chromatographic (GC) analysis. Individual plant roots were exposed in glass jars to 250 mL of metalaxyl-spiked nutrient media for 7 d. Plant roots were rinsed with distilled water after the 7-d exposure period. The plants were then placed in nonspiked nutrient media and allowed to grow for an additional 7 d in order 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 coffee cup with a hole cut into the bottom. Measured endpoints differed depending on the plant species due to differences in growth habit and size. Fresh weights were recorded before exposures, after 7 d exposure, and after the 7-d post-exposure period for all species evaluated. These measurements were used to calculate the fresh weight gains during the 7-d exposure and post-exposure periods. Growth, measured as the increase in length and branch production, was also recorded for parrotfeather. Chlorophyll fluorescence parameters were measured on intact canna and pickerelweed plants at the same time intervals using an OPTISCIENTES (Haverhill, MA) OS-500 modulated fluorometer. Specific chlorophyll fluorescence emission parameters measured included initial fluorescence (Fo), maximal fluorescence (Fm), steady state fluorescence (Fs), and maximal steady state fluorescence (Fms). Fv was calculated as Fm – Fo. The ratio of Fv to Fm is considered the quantum efficiency. Y (yield) was calculated as the difference between (Fms – Fs)/Fms. This is a measure of quantum efficiency under lighted, steady state conditions. Plants were acclimated to darkness for at least 20 min before Fo and Fm measurements were taken. All fluorescence measurements were taken at the second leaf from the youngest for both species. These measurements were not possible with sweetflag and parrotfeather because of the small size and morphology of their leaves.

These flurometric measurements are typically used to examine the physiological status of the photosynthetic apparatus and are based on the phenomenon that 3 to 5% of the light energy absorbed by chlorophyll pigments is re-emitted as fluorescence under normal conditions (Krause and Weis, 1984; Miles, 1990; Karukstis, 1991). Photosynthesis-inhibiting pesticides and other substances or environmental conditions that alter the overall bioenergetic status of the plant may induce characteristic changes in fluorescence emissions (Hipkins and Baker, 1986). The reader is referred to the previously mentioned resources and to 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. A completely randomized statistical design with four replications for each exposure concentration was used. All data were ranked and analyzed by analysis of variance (P = 0.05). Results were further analyzed using calculated least significant differences (LSD).

Uptake and Distribution

The uptake and distribution of metalaxyl by each plant species was evaluated using uniformly ring-labeled [¹⁴C]metalaxyl. This material was obtained from Novartis Crop Protection and had a reported chemical purity of 98.1% and radioactivity of 98.6%. The specific activity of the [¹⁴C] metalaxyl was 1.33 × 10⁶ Bq mg⁻¹. Approximately 5.33 × 10⁶ Bq of [¹⁴C] metalaxyl were dissolved overnight in 3.8 to 4.4 L of 10% Hoagland’s nutrient media. This was equivalent to a concentration of approximately 0.909 mg L⁻¹. Fourteen 250-mL sidearm vacuum flasks were each filled with 275 mL of the spiked nutrient media. Nonspiked nutrient media served as untreated controls. 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 (U.S. size no.) silicon stoppers with holes bored through the tops, and a slit along the side. Stoppers were wrapped around individual plant stems and sealed using Qubitac (Qubit, Kingston, ON, Canada) 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 [¹⁴C] CO₂ and volatile organic [¹⁴C] trap. The traps contained 15 mL of 0.5 M NaOH and 2 g of 20-60 mesh-activated charcoal (Sigma, St. Louis, MO), respectively. Two to four flasks containing the spiked nutrient media, but without plants, were also included as references. The untreated controls and 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 Conviron CMP3244 (Controlled Environments, Winnipeg, MB, Canada) environmentally controlled growth chamber (light intensity, 375 ± 25 μmol m⁻² s⁻¹ generated by fluorescent and incandescent lamps; photoperiod, 16 h light, 8 h dark; relative humidity, 60%; temperatures, 25°C light, 22°C 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—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/or tubers, and roots. Fresh weights for each individual section were recorded. Plant tissues were then 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, tissues were freeze-dried, weighed, and combusted in an R.J. Harvey (Hillsdale, NJ) Biological Oxidizer using a 3 min combustion cycle. The [¹⁴C]CO₂ generated by combustion was captured using an R.J. Harvey [¹⁴C]CO₂ trapping cocktail. The captured [¹⁴C]CO₂ content was analyzed using a Beckman (Fullerton, CA) LS 6500 Liquid Scintillation Counter. The [¹⁴C] recovery efficiency was determined by combusting (and analyzing as previously mentioned) 100 mg mannotol spiked with 0.37 Bq [¹³C]metalaxyl dissolved in acetone. The ratio of activity detected to activity added represented the recovery efficiency, and was used to adjust all values to 100%.

**RESULTS AND DISCUSSION**

** Toxicity**

Fresh mass production for sweetflag was only 35 and 48% of the controls at the 75 and 100 mg L⁻¹ treatment levels, respectively, after 7 d exposure (Fig. 1A). Fresh mass production for plants exposed to 75 mg L⁻¹ increased 13% during the 7-d post-exposure period. Statistical similarities of the 75 mg L⁻¹ treatments with the controls after the 7-d post-exposure period were probably an artifact due to variation in the plant response. The coefficient of variation for this treatment level was three times greater than that for the controls. Likewise, coefficients of variation for plants exposed to 100 mg L⁻¹ metalaxyl were also greater than three times those of the controls.

Metalaxyl did not affect growth of the canna and pickerelweed, measured as fresh mass production, during the 7-d exposure and post-exposure periods (Fig. 1B, D). Likewise, no reductions in PS II quantum efficiency measured as Fv/Fm or fluorescence yield were observed with either species. The range of values for each fluorometric parameter and species is listed in Table 1. While
Table 1. Range of values for fluorescence parameters measured on canna and pickerelweed. Values are unitless.

<table>
<thead>
<tr>
<th>Species</th>
<th>( F_0 ) $^\dagger$</th>
<th>( F_m ) $^\ddagger$</th>
<th>( Fv/Fm ) $^§$</th>
<th>( Fs ) $^¶$</th>
<th>( Fms ) $^#$</th>
<th>( Y ) $^{²²}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canna</td>
<td>286.7–340.5</td>
<td>1570.8–1754.3</td>
<td>0.792–0.824</td>
<td>223–286.5</td>
<td>817.8–1131</td>
<td>0.716–0.784</td>
</tr>
<tr>
<td>Pickerelweed</td>
<td>269–350.2</td>
<td>1493–1786.5</td>
<td>0.805–0.847</td>
<td>177.5–262.5</td>
<td>771.2–1023.5</td>
<td>0.723–0.791</td>
</tr>
</tbody>
</table>

$\dagger$ Initial fluorescence.  
$\ddagger$ Maximal fluorescence.  
§ \( Fv/Fm \) quantum efficiency.  
¶ Steady state fluorescence.  
# Maximal steady state fluorescence.  
$^{²²}$ \( Y = (Fms - Fs)/Fms \).  

these measurements showed no toxic effects on the plants, visual necrosis of both species exposed to 50, 75, and 100 mg L$^{-1}$ nutrient solution was apparent after 3 d exposure. This damage was initially characterized by brown, necrotic speckling of the lower leaves. Necrosis began at the leaf veins of the older leaves and spread throughout those leaves. After 7 d exposure, necrosis appeared on the tips of new leaves. The appearance of necrosis in the absence of effects on quantum efficiency indicates that the mode of action for toxicity was not related to PSII or processes directly interacting with PSII.

No reductions in fresh mass production were observed for parrotfeather during the 7-d exposure period (Fig. 1C). However, fresh mass production was statistically reduced 16, 20, and 24% at the 25, 75, and 100 mg L$^{-1}$ treatment levels, respectively, relative to the controls. The lack of reductions in the 50 mg L$^{-1}$ treatment group was probably due to natural variations in plant material. Plants in all treatment groups had similar fresh masses before exposure.

Measurements of stem growth were less sensitive than fresh mass production (Fig. 2A). Regardless of whether branch length was included with primary stem length increases, no statistically significant reductions in growth were observed. Likewise, no reductions in axillary branch production were observed during the experiment (Fig. 2B).

These results agree with those of Novartis (Novartis Crop Protection Environmental Safety Database, unpublished data, 1998). They reported 14-d EC50 values of 85 mg L$^{-1}$ for frond production in duckweed (\textit{Lemna gibba} L.) and 140 mg L$^{-1}$ based on mean standing crop of \textit{Selenastrum capricornutum} Printz. Typical standardized plant toxicity testing protocols for water column contaminants utilize these species (Strois, 1990; American Public Works Association, American Water Works Association, and Water Environment Federation, 1992, p. 8.39–8.42; American Society for Testing and Materials, 1994).

Based on these results, little caution is warranted when using these ornamentals for the phytoremediation of metalaxyl. However, one must realize that these tests represented a worst-case scenario where other substrates capable of reducing bioavailability were not present. Under natural conditions in the field, significant portions of the pesticide may be sorbed to substrates or degraded by microorganisms and sunlight. In the case of a gravel-based phytoremediation system designed for removing pesticides from water, some caution is still warranted due to the expected pesticide concentrations. Expected metalaxyl concentrations in 568 L of rinse water were calculated to range from 4.7 to 46.8 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 between rinses. These concentrations would be much greater at higher application rates.

Uptake and Distribution

Sweetflag

The [\(^{14}\text{C}\)]metalaxyl activity in the exposure solutions decreased with time (Table 2). These reductions were 4, 4, 10, and 16%, respectively, after 1, 3, 5, and 7 d of exposure. The amount of activity remaining in the exposure solution was inversely related to the cumulative volume of water used by the plant (Table 3).

Nearly all of the [\(^{14}\text{C}\)]metalaxyl removed from the exposure solution was detected in the plants. After 1, 3, 5, and 7 d exposure, 3, 5, 8, and 18% of the total activity added, respectively, was detected in the whole plants. The mass balance for [\(^{14}\text{C}\)] was good throughout the test, with unmeasured label never exceeding 2%
The accumulation of activity in whole plants was directly related to the amount of water used by those plants (Table 3).

Analysis of the dissected plant tissues revealed significant accumulation of \(^{14}\text{C}\) primarily in the leaves, and to a lesser extent in the roots (Table 2). No accumulation was seen in the rhizomes, indicating that they serve primarily as a pathway for acropetal transport.

### Canna

The \(^{14}\text{C}\)metalaxyl activity in the exposure solutions decreased with time (Table 4). These reductions were 4, 31, 48, and 60%, respectively, after 1, 3, 5, and 7 d of exposure. The amount of label remaining in the exposure solution was inversely related to the cumulative amount of water used by the plants (Table 3).

Nearly all of the \(^{14}\text{C}\)metalaxyl removed from the exposure solution was detected in the plants. After 1, 3, 5, and 7 d exposure, 13, 29, 37, and 54% of the total activity added, respectively, was detected in the whole plants harvested after 1 d of exposure. This excess may have resulted from the lower recovery efficiency of samples, since all data were adjusted to 100% recovery corrections based on 93% recoveries would artificially inflate total recoveries and the mass balance. The mass

### Table 2. Percent† distribution of total \(^{14}\text{C}\) activity initially present in exposure solutions with or without sweetflag.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental vessels with plants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solution§</td>
<td>0.96</td>
<td>0.96</td>
<td>0.90</td>
<td>0.84</td>
</tr>
<tr>
<td>Impurity¶</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Roots</td>
<td>0.011</td>
<td>0.013</td>
<td>0.018</td>
<td>0.018</td>
</tr>
<tr>
<td>Rhizomes</td>
<td>0.003</td>
<td>0.004</td>
<td>0.006</td>
<td>0.006</td>
</tr>
<tr>
<td>Leaves</td>
<td>0.012</td>
<td>0.032</td>
<td>0.06</td>
<td>0.029</td>
</tr>
<tr>
<td>Unaccounted#</td>
<td>0.004</td>
<td>+0.019</td>
<td>0.006</td>
<td>+0.025</td>
</tr>
<tr>
<td>Reference vessels with no plants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solution§</td>
<td>0.94</td>
<td>0.94</td>
<td>0.95</td>
<td>0.95</td>
</tr>
<tr>
<td>Impurity¶</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

† Fractional form.
‡ CV = coefficient of variation.
§ Solution = metalaxyl in exposure solution.
¶ Impurity = unidentified impurity and/or metabolite in exposure solution determined by high performance liquid chromatography (HPLC).
# + = activity in excess of what was originally added.

### Table 3. Regression equations relating cumulative water used to percent activity remaining in solution and percent accumulated in plants.

<table>
<thead>
<tr>
<th>Species</th>
<th>Regression equation</th>
<th>(\text{Percent remaining in solution})</th>
<th>(\text{Percent accumulated in plants})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweetflag</td>
<td>(%) remaining = (-0.00153 \times (\text{mL water used}) + 1.00441)</td>
<td>9.08 (\times) (10^{-5})</td>
<td>4.7 (\times) (10^{-5})</td>
</tr>
<tr>
<td>Canna</td>
<td>(%) remaining = (-0.00117 \times (\text{mL water used}) + 1.01696)</td>
<td>2.73 (\times) (10^{-5})</td>
<td>7.51 (\times) (10^{-5})</td>
</tr>
<tr>
<td>Parrotfeather</td>
<td>(%) remaining = (-0.00145 \times (\text{mL water used}) + 1.00319)</td>
<td>3.92 (\times) (10^{-5})</td>
<td>6.24 (\times) (10^{-5})</td>
</tr>
<tr>
<td>Pickerelweed</td>
<td>(%) remaining = (-0.00111 \times (\text{mL water used}) + 1.00117)</td>
<td>2.44 (\times) (10^{-5})</td>
<td>3.44 (\times) (10^{-5})</td>
</tr>
</tbody>
</table>

### Table 4. Percent† distribution of total \(^{14}\text{C}\) activity initially present in exposure solutions with or without canna.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental vessels with plants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solution§</td>
<td>0.96</td>
<td>0.96</td>
<td>0.90</td>
<td>0.84</td>
</tr>
<tr>
<td>Impurity¶</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Roots</td>
<td>0.029</td>
<td>0.024</td>
<td>0.026</td>
<td>0.026</td>
</tr>
<tr>
<td>Tubers</td>
<td>0.012</td>
<td>0.010</td>
<td>0.013</td>
<td>0.013</td>
</tr>
<tr>
<td>Stems</td>
<td>0.008</td>
<td>0.009</td>
<td>0.009</td>
<td>0.009</td>
</tr>
<tr>
<td>Leaves</td>
<td>0.085</td>
<td>0.250</td>
<td>0.325</td>
<td>0.489</td>
</tr>
<tr>
<td>Unaccounted#</td>
<td>+0.104</td>
<td>-0.007</td>
<td>-0.097</td>
<td>-0.057</td>
</tr>
<tr>
<td>Reference vessels with no plants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solution§</td>
<td>0.99</td>
<td>0.98</td>
<td>0.99</td>
<td>0.98</td>
</tr>
<tr>
<td>Impurity¶</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

† Fractional form.
‡ CV = coefficient of variation.
§ Solution = metalaxyl in exposure solution.
¶ Impurity = unidentified impurity and/or metabolite in exposure solution determined by high performance liquid chromatography (HPLC).
# + = activity in excess of what was originally added.
balance for $^{14}$C was generally good throughout the test, with unmeasured label ranging from 0.7 to 9.7% (Table 4). The total amount of activity accumulated within each plant was directly related to cumulative water use by each plant (Table 3).

Analysis of the dissected plant tissues revealed significant accumulations of $^{14}$C in the leaves (Table 4). No accumulation was seen in the stems and roots, indicating that they serve only as a pathway for acropetal transport.

**Parrotfeather**

The $^{14}$C-metalaxyl activity in the exposure solutions decreased with time (Table 5). These reductions were 5, 9, 19, and 31%, respectively, after 1, 3, 5, and 7 d of exposure. The amount of activity remaining in the exposure solution was inversely related to cumulative water use by each plant (Table 3).

Nearly all of the $^{14}$C-metalaxyl removed from the exposure solution was detected in the plants. After 1, 3, 5, and 7 d exposure, 10, 24, 32, and 44% of the total activity added, respectively, was detected in the whole plants. The mass balance for $^{14}$C was good throughout the test, with unmeasured label never exceeding 3.3% (Table 6). The accumulation of activity within plants was directly proportional to the cumulative amount of water used by the plants (Table 3). Analysis of the dissected plant tissues revealed significant accumulations of $^{14}$C primarily in the leaves (Table 5). Very little accumulation was seen in the stems and roots, indicating that they serve only as a pathway for acropetal transport.

### Table 5. Percent† distribution of total $^{14}$C activity initially present in exposure solutions with or without parrotfeather.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n = 3$</td>
<td>CV</td>
<td>$n = 3$</td>
<td>CV</td>
</tr>
<tr>
<td>Solution§</td>
<td>0.95</td>
<td>0.04</td>
<td>0.91</td>
<td>0.01</td>
</tr>
<tr>
<td>Impurity¶</td>
<td>0.01</td>
<td>0.04</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Roots</td>
<td>0.004</td>
<td>0.876</td>
<td>0.009</td>
<td>0.00</td>
</tr>
<tr>
<td>Root stems</td>
<td>0.001</td>
<td>0.00</td>
<td>0.002</td>
<td>0.00</td>
</tr>
<tr>
<td>Stems</td>
<td>0.004</td>
<td>0.250</td>
<td>0.008</td>
<td>0.125</td>
</tr>
<tr>
<td>Leaxes</td>
<td>0.024</td>
<td>0.00</td>
<td>0.089</td>
<td>0.081</td>
</tr>
<tr>
<td>Unaccounted#</td>
<td>0.007</td>
<td>–</td>
<td>0.028</td>
<td>–</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reference vessels with no plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution§</td>
</tr>
<tr>
<td>Impurity¶</td>
</tr>
</tbody>
</table>

† Fractional form.
§ Solution = metalaxyl in exposure solution.
¶ Impurity = unidentified impurity and/or metabolite in exposure solution determined by high performance liquid chromatography (HPLC).
# + = activity in excess of what was originally added.
mainly in the leaf blades throughout the experimental period (Table 6).

In all cases, the unidentified impurity–metabolite levels in the flasks remained constant. As a result, the assumption that the activity detected in the plants was due primarily to uptake of parent metalaxyl was valid. Metalaxyl levels within reference flasks containing no plants also remained relatively constant throughout the exposure periods (Tables 2, 4–6). No significant amounts of $^{14}$C were detected in the NaOH scrubbers, indicating little loss due to mineralization in the rhizosphere. Losses due to volatilization were probably not significant due to metalaxyl’s low vapor pressure of $5.62 \times 10^{-6}$ mm Hg and comparatively high water solubility of 8.4 g L$^{-1}$ (Wauchope et al., 1992). In general, the $^{14}$C mass balance for all of these experiments was good, with under-accounted-for masses never exceeding 11% and over-accounted-for masses never exceeding 11% of the original activity added to the media. 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.

Although these results indicate that the ornamental species may be useful for removing metalaxyl from the soil solution and contaminated water, it is important to realize that in these scenarios, all of the pesticide was in solution. This might be the case under engineered conditions such as with constructed wetlands designed to maximize root contact with the contaminated water. However, under most field conditions, competing processes such as sorption to soils and organic materials and degradation due to photolysis and microbial activity would probably reduce the amount of metalaxyl available for uptake by the plants.

One question that this research did not approach is the fate of metalaxyl once it is in the plants. This was not possible because of the destructive nature of combating the samples. However, it is likely that some metabolism occurred. Plants have been shown to metabolize metalaxyl by ring methyl hydroxylation, aryl hydroxylation, ester cleavage, O-dealkylation, and N-dealkylation (Businelli et al., 1984; Cohen and Coffey, 1986; Owen and Donzel, 1986; Cole and Owen, 1987).

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

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