Ecological Engineering 47 (2012) 254-263

Contents lists available at SciVerse ScienceDirect

FLSEVIER





journal homepage: www.elsevier.com/locate/ecoleng

Remediation of metalaxyl, trifluralin, and nitrate from nursery runoff using container-grown woody ornamentals and phytoremediation areas

Aaron L. Warsaw^{a,b}, R. Thomas Fernandez^{a,*}, Dan R. Kort^a, Bert M. Cregg^a, Brad Rowe^a, Chris Vandervoort^c

^a Department of Horticulture, Plant & Soil Sciences Building, Michigan State University, East Lansing, MI 48824, USA

^b 39600 E. 400 North RD., Bellflower, IL 61724, USA

^c Department of Plant Pathology, Center for Integrated Plant Systems, Michigan State University, East Lansing, MI 48824, USA

ARTICLE INFO

Article history: Received 28 January 2012 Received in revised form 9 June 2012 Accepted 23 June 2012 Available online 26 July 2012

Keywords: Fungicide Herbicide Nursery production Pesticide Water management

ABSTRACT

Two commonly container-produced woody ornamentals, Salix alba L. 'Britzensis' and Sambucus nigra L. 'Aurea', were grown in aquatic phytoremediation areas (PAs) to evaluate phytoremediation of trifluralin (2004 and 2005), metalaxyl (2006), and NO₃⁻-N (2004-2006) from nursery runoff. The use of container-grown ornamentals for phytoremediation would prevent land area used in production from being converted into wetlands or vegetated filter strips. Twelve nursery beds (NBs) were constructed and graded to allow runoff collection in a small basin at the west end of each production area. Runoff from each NB drained into a PA that contained one of three treatments: (1) 20 plants of S. alba 'Britzensis' grown in containers, (2) 20 plants of S. nigra Aurea' grown in containers, or (3) a PA with no containers and plants serving as a control. Samples of runoff from NBs and water leaving PAs were collected for trifluralin or metalaxyl and NO₃⁻-N analysis. Phytoremediation areas with container-grown plants remediated trifluralin, metalaxyl, and NO₃⁻-N in runoff water to the same extent as PAs without plants. When data from all treatments were pooled and water exiting PAs was compared to runoff from NBs, PAs were effective in remediating runoff. In 2004 and 2005, overall average trifluralin quantity was reduced by 72% (7.32 \pm 0.67 mg to 2.03 \pm 0.29 mg) and 94% (from 7.81 \pm 0.86 mg to 0.51 \pm 0.07 mg) as runoff moved through PAs. The overall average metalaxyl quantity was reduced by 48% (34.32 ± 7.98 mg to 17.69 ± 2.78 mg) as runoff moved through PAs. Nitrate-nitrogen quantities leaving PAs were the same or lower compared to NO₃⁻-N captured from NBs depending on sample day, with the greatest reduction of 98% (373-6.00 mg) on 14 July 2006. Plants grown in PAs reached marketable size during the experiment. Using PAs with or without container-grown plants as a component of the PA system would reduce environmental contamination from runoff containing metalaxyl, trifluralin, and NO₃⁻-N, and would reduce harmful effects to aquatic organisms from runoff containing trifluralin or NO_3^- -N.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Concerns about the contamination of water resources from chemicals in nursery runoff have increased as awareness of chemical impacts on environmental and human health has risen. High levels of irrigation, fertilizer, and pesticide inputs are common in the production of container-grown plants. Runoff from frequent irrigation applications carries fertilizers and other agricultural chemicals off production surfaces where they may enter surrounding water resources. Runoff quantity is increased when production surfaces are semi-impermeable to impermeable to water infiltration, resulting in sheet flow across production areas. Semi-impermeable surfaces also decrease the time available for water to infiltrate into substrate/soil below the production surface where chemicals can degrade or bind to soil particles before entering water resources.

Granular pre-emergence herbicides are often applied multiple times during the growing season for weed control in container nurseries (Gilliam et al., 1992). Granular herbicides are applied by broadcasting them over production areas. Container size and spacing at the time of application affect non-target losses that occur when applied pesticides land on production areas and in between containers. Gilliam et al. (1992) reported non-target losses of granular herbicides were 51% and 80% when applied to empty

^{*} Corresponding author at: A216 Plant & Soil Science Building, Department of Horticulture, East Lansing, MI 48824-1325, USA. Tel.: +1 517 355 5191x1336; fax: +1 517 353 0890.

E-mail address: fernan15@msu.edu (R. Thomas Fernandez).

^{0925-8574/\$ -} see front matter © 2012 Elsevier B.V. All rights reserved. http://dx.doi.org/10.1016/j.ecoleng.2012.06.036

2.8-L containers spaced 20 cm and 30 cm on center, respectively. Additionally, drift can cause liquid pesticide applications to miss intended targets. Briggs et al. (2002a) reported 9.1% of applied granular formulation and 7.3% spray formulation of the herbicide isoxaben were detected in runoff from nursery beds. Losses of 0.5% the amount applied for both granular and spray formulations of the herbicide trifluralin have also been detected (Briggs et al., 2002a).

A common method of fertilizing container-grown plants is the use of controlled release fertilizers (CRFs). Fare et al. (1994) reported NO₃⁻-N losses of 63%, 56%, and 47% of total applied N when 13 mm of irrigation was applied in one, two, and three cycles, respectively. Yeager and Cashion (1993) reported NO₃⁻-N concentrations from CRFs in runoff periodically exceeded the 10 mg L⁻¹ federal drinking water standard (USEPA, 2009). Phosphorus losses from container substrates ranging from 8% to 27% have been reported by Warren et al. (1995). In Florida the EPA is proposing 0.35 mg L^{-1} nitrate-nitrite concentration limits as a protective criterion for aquatic life in Florida's springs and clear streams (USEPA, 2010). The EPA is also proposing nutrient criteria for canals in the Florida Department of Environmental Protection's South Florida bioregion for the following parameters: chlorophyll a (4.0 μ gL⁻¹), total phosphorus (0.042 mgL⁻¹), and total nitrogen (1.6 mg L^{-1} ; USEPA, 2010). Total nitrogen and total phosphorus proposed criteria for streams are 0.824 mg L⁻¹ to $1.798 \text{ mg } \text{L}^{-1}$ and $0.043 \text{ mg } \text{L}^{-1}$ to $0.739 \text{ mg } \text{L}^{-1}$, depending on nutrient watershed region (USEPA, 2010). Nitrate-nitrogen concentrations of 10 mg L^{-1} can adversely affect freshwater invertebrates (Eulimnogammarus toletanus, Echinogammarus echinosetosus, Cheumatopsyche pettiti, and Hydropsyche occidentalis), fishes (Oncorhynchus mykiss, Oncorhynchus tshawytscha, and Salmo clarki), and amphibians (Pseudacris triseriata, Rana pipiens, Rana temporaria, and Bufo bufo) during long term exposures, with a recommended maximum level for protecting sensitive freshwater animals from NO_3^- – N pollution of 2 mg L⁻¹ (Camargo et al., 2005).

Nurseries are increasingly recycling water for irrigation by capturing runoff in containment ponds or retention basins. However, production concerns arise because recycled irrigation can contain herbicides and nutrients from production runoff (Camper et al., 1994; Riley et al., 1994). While residual amounts of herbicides can be effective against sensitive weeds (Skimina, 1992), phytotoxicity can become a problem to herbicide-sensitive plants (Bhandary et al., 1997a,b). Vegetated treatment systems (constructed

wetlands and vegetated ditches), vegetated filter strips, and grassed waterways have been successfully used for reducing pesticide and nutrient movement in runoff from agricultural land and container nurseries (Asmussen et al., 1977; Magette et al., 1989; Dillaha et al., 1989; Mickelson and Baker, 1993; Briggs et al., 1998; Maillard et al., 2011; Stehle et al., 2011; Tanner and Sukias, 2011). Stehle et al. (2011) conducted a meta-analysis using data from 24 scientific articles on vegetated treatment systems (VTS) and concluded that VTS are an effective risk mitigation method for reducing pesticide exposure levels in downstream surface waters.

Although successful, the aforementioned systems require land area be set aside for the purpose of runoff remediation. An alternative to removing valuable land from production for the construction of wetlands or vegetated filter strips is to construct phytoremediation areas (PAs) that use container-grown semiaquatic plants to remediate runoff (Fernandez et al., 1999; Baz and Fernandez, 2002). Runoff is directed through PAs to improve runoff water quality before entering the surrounding environment or retention ponds and the container-grown plants can be sold upon attaining marketable size. Phytoremediation areas in this experiment were located next to nursery beds to immediately capture runoff leaving nursery beds. This arrangement coincides with Stehle et al. (2011) conclusion that it is vital to locate VTS as close as possible to the areas where pesticides are applied. Phytoremediation is defined as the use of plants to remove pollutants from the environment or to render them harmless (Cunningham and Berti, 1993; Raskin et al., 1994). Some advantages of phytoremediation over mechanical treatments are that phytoremediation is in situ, passive, solar driven, and therefore, costs 10-20% of mechanical treatments (Susarla et al., 2002). For pesticides and herbicides, evidence suggests that metabolic end products of biodegradation of these compounds may be bound irreversibly by the plant (Komossa et al., 1995; Trapp and MacFarlane, 1995; Field and Thurman, 1996). [2,6-dinitro-N,N-dipropyl-4-Trifluralin

(trifluoromethyl)benzenamine] is a selective, pre-emergence herbicide used to control annual grasses and broadleaf weeds in a variety of crops. Trifluralin is not highly water soluble at 24 mg L^{-1} at $27 \,^{\circ}\text{C}$ and is strongly adsorbed on soils (Koc = $7.00 \times 10^3 \text{ g mL}^{-1}$; Table 1). Trifluralin is subject to degradation by soil microorganisms, volatilization, and UV light. Trifluralin is toxic to fish and other aquatic organisms with LC₅₀ values for *Daphnia magna* (water flea) and *O. mykiss* (Rainbow trout) listed in Table 1. Other properties

Table 1

Selected chemica	l properties fo	r the	herbicide	trifluralin	and th	e fungicide	metalaxyl.
------------------	-----------------	-------	-----------	-------------	--------	-------------	------------

Property	Chemical					
	Trifluralin	Source	Metalaxyl	Source		
Chemical name	2,6-Dinitro-N,N-dipropyl-4- (trifluoromethyl)benzenamine	b	N-(2,6-Dimethylphenyl)-N- (methoxyacetyl)-alanine methyl ester	a		
Water solubility	24 mg L ^{−1} at 27 °C	b	$8.40 imes10^3$ mg L $^{-1}$ at 22 °C	а		
Density	1.36 at 22 °C	b	1.20 at 20 °C	а		
Log Kow	5.34	b	1.65	a		
Кос	$7.00 \times 10^3 \mathrm{g}\mathrm{mL}^{-1}$	b	$30-284 \mathrm{g}\mathrm{mL}^{-1}$	a		
Vapor pressure	4.58 × 10 ^{−5} mmHg at 25 °C	b	5.62×10^{-6} mmHg at $25 \degree C$	а		
Soil half-life	45-60 days	с	Approx. 40 days	а		
LC ₅₀ <i>D. magna</i> (water flea) for 48 h exposure	$560 \mu g L^{-1}$	d	$5.15 \times 10^4 \mu g L^{-1}$	e		
LC ₅₀ Oncorhynchus mykiss (Rainbow trout) for 96 h exposure	41 μg L ⁻¹	d	$1.30\times 10^5 \ \mu g L^{-1}$	е		

^a Toxicology Data Network (2003).

^b Toxicology Data Network (2002).

^c U.S. Department of Agriculture (1990).

^d U.S. Department of Interior, Fish and Wildlife Service (1980).

^e U.S. Environmental Protection Agency (2007).

of trifluralin are listed in Table 1. It is not significantly absorbed or translocated in plants grown in soils treated with trifluralin and residues will occur on root tissues which have directly contacted trifluralin (USDA Soil Conservation Service, 1990). However, Li et al. (2002) showed that trifluralin uptake, metabolism, and the formation of bound residues occurred in *Lolium multiflorum* Lam. growing in solutions containing trifluralin.

Metalaxyl [*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)alanine methyl ester] is a systemic benzenoid fungicide commonly used for controlling the pathogens *Phytophthora* and *Pythium* spp. (Kimmel et al., 1986; Wilson et al., 2001). Metalaxyl can be applied as a foliar spray, soil treatment, or as a seed treatment for downy mildews (Kimmel et al., 1986). Metalaxyl is highly water soluble at 8.40×10^3 mg L⁻¹ at 22 °C and is highly to moderately mobile in soils with Koc values ranging from 30 to 284 g mL⁻¹ (Table 1). The LC₅₀ of metalaxyl to D. magna (water flea) and O. mykiss (Rainbow trout) along with other properties of metalaxyl is listed in Table 1. Metalaxyl has been detected in runoff (Dunn et al., 2011; Maillard et al., 2011), and Briggs et al. (2002b) reported that 25% of applied metalaxyl was recovered in runoff at a commercial container nursery. Metalaxyl uptake by plants growing in an aqueous nutrient solution (10% Hoagland's nutrient solution; Hoagland and Arnon, 1938) has been documented in Acorus gramineus Sol. Ex Aiton, Canna hybrida L. 'Yellow King Humbert', Myriophyllum aquaticum (Vell.) Verde., Pontederia cordata L., and Typha latifolia L. (Wilson et al., 2000, 2001). Furthermore, metalaxyl is metabolized by plants (Businelli et al., 1984; Cohen and Coffey, 1986; Owen and Donzel, 1986; Cole and Owen, 1987).

Fernandez et al. (1999) showed that the semiaquatic herbaceous perennials *Canna* × *Typha latifoligeneralis* L.H. Bail., *Pontaderia cordata* L., and *Iris* L. × 'Charjoys Jan' can be useful in PAs for remediation of the herbicide oryzalin. Baz and Fernandez (2002) reported that the woody ornamentals *Itea virginica* L. 'Sprich' and *Salix alba* L. showed potential for use in the phytoremediation of oryzalin at 4 mg L⁻¹ but not isoxaben at 4 mg L⁻¹ based growth and photosynthetic responses. Further research identifying ornamental plant species for use in a container-grown phytoremediation system is needed. The objective of this study was to characterize and evaluate the effectiveness of container-grown *S. alba* 'Britzensis' and *Sambucus nigra* 'Aurea' grown in PAs for reducing the concentration of trifluralin, metalaxyl, and NO₃⁻–N from nursery production surface runoff.

2. Materials and methods

2.1. Plant material

Two species of container-grown woody ornamentals were grown in PAs. S. alba 'Britzensis' is a male willow cultivar grown primarily for its orange to reddish stems, which provide winter interest (Dirr Michael, 2009) The species grows naturally along wet areas. Kuzovkina and Volk (2009) reported that the genus Salix exhibits many essential agronomical, physiological, and ecological characteristics that make it a practical choice for use in phytoremediation including: high growth rate, tolerance of high density planting, high rates of transpiration, high nutrient uptake and nitrogen use, and resistance to chemical contaminants. High ornamental value was listed as an essential characteristic under land reclamation uses by Kuzovkina and Volk (2009). Ornamental value was a vital characteristic of plants used in our study because plants used for phytoremediation must also be commercially valuable when marketable size is attained. S. alba has also shown promise for use in phytoremediation of the herbicide oryzalin (Baz and Fernandez, 2002). S. nigra 'Aurea' is a multi-stemmed shrub or small Tree 3–4.5 m tall with bright golden young foliage, fragrant white flowers, and ornamental black fruit that grows best in consistently moist soils (Dirr Michael, 2009).

Plants were received from a commercial nursery as 5.7-cm potted liners for *S. alba* 'Britzensis' and 10-cm potted liners for *S. nigra* 'Aurea' in August 2002. They were transplanted into 15.6-L containers during the week of Sept 9, 2002. Plant size is one component of determining salability of container grown ornamentals. Therefore plant height and width were measured periodically during each year of the study to assess plant marketability.

2.2. Nursery beds and phytoremediation areas

Twelve $3 \text{ m} \times 6 \text{ m}$ nursery beds (NBs) were constructed at the Michigan State University Horticultural Teaching and Research Center in Holt, Michigan (Fig. 1). Nursery beds were oriented east to west with collection reservoirs and PAs at the west end of NBs. The surface of the beds were lined with 6 mil black polypropylene plastic and covered with a black landscape fabric. Nursery beds were separated by 3.66 m to minimize effects of irrigation and pesticide drift. The beds were graded toward the center and west end to direct runoff into an excavated collection reservoir $(243 \text{ cm} \times 61 \text{ cm} \times 14 \text{ cm})$ at the west end of each NB. Collection reservoirs were constructed with a wooden frame and lined with 45 mil black polypropylene pond liner. Reservoirs were sloped to one side so that runoff could be pumped out of the reservoir more easily for volume measurement. To the west side of each runoff collection reservoir, an aquatic plant production area was established as a phytoremediation area (PA). Twelve PAs $(305 \text{ cm} \times 122 \text{ cm} \times 14 \text{ cm})$ were constructed with a wooden frame and lined with 45 mil black polypropylene pond liner. A drain connected to 2.54 cm inner diameter PVC pipe was installed in the west side of the PA to direct water exiting PAs away from the production site and to prevent PAs from overflowing. Precipitation was not excluded from NPAs and PAs but was recorded by an on-site Michigan Automated Weather Network weather station.

Following an irrigation or precipitation event runoff from the NB would flow by gravity into the NB collection reservoir. When the collection reservoir was full, runoff would flow over the west wall of the collection reservoir into the PA. Phytoremediation areas contained water including precipitation and runoff from NBs that entered from previous irrigation events.

2.3. Runoff collection

Before runoff collection on the day of chemical applications, all NB collection reservoirs were pumped empty to remove runoff from previous irrigation applications or precipitation that had not yet flowed into the PA. One hour after chemical application (day 0), irrigation was applied to NBs and runoff in NB reservoirs was collected 30 min after irrigation termination. All runoff from a single irrigation application was captured in the NB runoff collection reservoir because a single irrigation event did not apply enough water to fill the collection reservoir to the level at which runoff flowed over the west wall into the PA. To measure runoff volume, runoff from each NB reservoir was pumped from the NB collection reservoir using an electric pump into a collection container (plastic 170-L garbage can). The height of runoff in the collection container was measured to the nearest centimeter. The collection container was calibrated to measure volume by adding water to the container in 10L increments and measuring the height of the water in the collection container. The height to volume relationship was fitted by linear regression ($r^2 = 0.998$) and the resulting equation was used to calculate runoff volumes from the height of runoff in the collection container. Following runoff volume

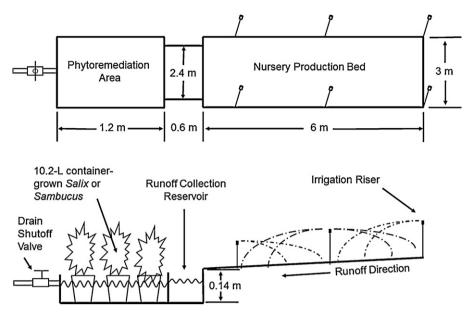


Fig. 1. Overhead and side view of one nursery bed (NB), runoff collection reservoir, and phytoremediation area (PA). Phytoremediation areas contained 20 potted Salix, 20 potted Sambucus, or no pots nor plants (control). Nursery beds were sloped to the center (not shown in diagram) and towards the runoff collection reservoir. Runoff flowed by gravity from the NB into the runoff collection reservoir. When the runoff collection reservoir was full runoff flowed over the back wall and into the PA. Container-grown woody ornamentals on NBs are not depicted in the figure to simplify presentation. Important dimensions are shown but figure is not drawn to scale.

measurement, runoff samples were taken and stored in glass bottles for trifluralin, metalaxyl and/or nitrate analysis.

Drains in each PA were closed prior to pumping the collected runoff into the PA so that the volume of water leaving the PA could be measured. The collected runoff was then pumped from the collection container into the PA which usually caused the water level in the PA to rise higher than the closed drain. Next, water was pumped out of the PA into the collection container until the water level in the PA was at the same level as the bottom of the closed drain. This volume was recorded and corresponded to the water volume that would have passed through the PA and into the surrounding environment if the drain had been open. If the runoff volume pumped into the PA from the NB collection basin did not raise the water level in the PA to the bottom of the drain, which occasionally happened during hot periods or prolonged dry periods as plants in PAs transpired and water from PAs evaporated, a volume of zero was recorded for water volume leaving the PA. A water sample was collected for trifluralin or metalaxyl and nitrate analysis and stored in a glass bottle. The drain on the PA was then opened and the water in the collection container was returned to the PA. All collected samples were stored on ice in a cooler at the experiment site, transported to the laboratory, and refrigerated at 4 °C until extraction and analysis.

2.4. Experimental design

The experiment used a completely randomized design with three treatments replicated four times. There were 12 PAs and each PA was one treatment replicate. Treatments were (1) 20 plants of *S. alba* 'Britzensis' grown in 15.6-L containers in a PA, (2) 20 plants of *S. nigra* 'Aurea' grown in 15.6-L containers in a PA, and (3) a control without containers and plants in the PA. A mixture of common container-grown species in 7.3-L, 10.2-L, and 15.6-L nursery containers size, and container arrangement the same in each NB. Plants were grown in an 85% pine bark: 15% peat moss (vol:vol) potting substrate.

Irrigation applications were scheduled with a Rain Bird ESP-12LX Plus controller (Rain Bird Corporation; Azusa/Glendora, CA, United States). Irrigation to each NB was controlled by a solenoid valve. Irrigation was applied to each NB through six Toro 570 Shrub Spray Sprinklers (The Toro Company; Riverside, CA, United States) mounted on 1.3 cm diameter risers at a height of 0.66 m. Emitter layout consisted of two 180 degree emitters and four 90 degree emitters per NB, all with 2.44 m radius distance of throw. Emitters were spaced 2.44 m apart to provide head to head coverage (100% overlap). Emitters were positioned on the outside edges of each NB with all irrigation directed into the NB. Irrigation was applied for 40 min to provide 2.5 cm of water based on a survey of growers in the southeastern United States who replied that they normally watered for approximately 1 h intending to apply 25 mm d⁻¹ of water (Fare et al., 1992) and from a study by Beeson (2006) who used a control treatment of 18 mm d⁻¹ to irrigate 11.4-L containergrown landscape shrubs. Irrigation was initiated between 0700 HR and 0800 HR each day.

2.5. Chemical applications

Trifluralin phytoremediation was evaluated in 2004 and 2005. Trifluralin was applied as Snapshot[®] 2.5TG (Dow AgroSciences LLC, Indianapolis, IN, United States) at a rate of 0.347 g active ingredient m⁻² (3.37 kg active ingredient ha⁻¹) or 6.25 g active ingredient applied per NB. Three applications of trifluralin were made during 2004 (26 May, 14 July, and 24 August) and 2005 (23 May, 11 July, and 30 August) to replicate in time and examine any seasonal differences. One water sample was taken from each NB runoff collection reservoir and PA on the day of application (day 0) and at 1, 2, and 4 days after application (DAA) to measure trifluralin concentration and quantity.

Metalaxyl phytoremediation was evaluated in 2006. Metalaxyl was applied as Subdue Maxx[®] (Syngenta Crop Protection, Inc., Greensboro, NC, United States) as a foliar spray at a rate of 0.036 g active ingredient m⁻² (0.347 kg active ingredient ha⁻¹) or 0.646 g active ingredient per NB. Three applications of metalaxyl were made during the 2006 experiment: 10 July, 31 July, and 5 September. One sample was taken from each NB runoff collection reservoir and PA on the day of application (day 0) and at 1, 2, 4, and 8 DAA to measure metalaxyl concentration and quantity.

In 2004 and 2005, plants grown on NBs were fertilized on 14 May with a 17.0N-3.1P-6.6K CRF with micronutrients with a release period of 5–6 months at 27 °C (Acid Special 5–7 Month Nursery Fertilizer, Wilbro Inc., Norway, SC, United States). Fertilizer was topdressed at a rate of 15 g per 7.6-L container (22 containers per NB) and 26 g per 10.2-L container (38 containers per NB). Total applied N per NB was 224g and applied at a rate of 12.4gm⁻² (121 kg ha⁻¹). In 2006, plants grown on NBs were fertilized on 5 June with a 17.0N-3.5P-6.6K CRF with micronutrients with a release period of 4 months at 27 °C (HFI Topdress Special, Harrell's Inc., Lakeland, FL, United States). Fertilizer was topdressed to containers at a rate of 26 g per 10.2-L container (66 containers per NB). Total applied N per NB was 293 g and applied at a rate of 16.3 g m^{-2} (157 kg ha⁻¹). In 2004, 2005, and 2006 *S. alba* 'Britzensis' and *S. nigra* 'Aurea' grown in 15.6-L containers in PAs were not fertilized and only received nutrients from NB runoff. Nitrate-nitrogen quantities and concentrations were measured three times during each year of the experiment, at 4 DAA of each trifluralin or metalaxyl application. One runoff sample was taken from each NB runoff collection reservoir and one water sample was taken from each PA.

2.6. Metalaxyl extraction

A 200 mL runoff sample was filtered to remove debris or sediments (Whatman Grade number 4 filter paper, Whatman International Ltd., UK) and adjusted to pH 2 with diluted hydrochloric acid. Extraction columns, Speedisk[®] H₂O-Phobic DVB Column (J.T. Baker A Division of Mallinckrodt Baker, Inc., Phillipsburg, NJ, United States) were prepared by adding 2 mL deionized water and allowed to soak the solid phase for 2 min. Next, 2 mL of methanol was slowly aspirated through the column under vacuum two times, followed by 2 mL water and 2 mL water at pH 2. Water samples were then aspirated through the column under vacuum and allowed to dry. Samples were then eluted from the column with 2 mL methanol under vacuum. Extracts were analyzed using a Hewlett Packard 6890 Gas Chromatograph (Hewlett Packard Co., Wilmington, DE, United States) connected to an Agilent 5973 Network Mass Selective Detector (Agilent Technologies, Inc., Santa Clara, CA, United States). A ZB-5MS column (30 m by 0.25 mm i.d. with 0.25 µm film) was used (Agilent Technologies, Inc.). Initial column temperature was 100 °C, ramping 7 °C min⁻¹ to 290 °C, followed by an increase of 10 °C min⁻¹ to 305 °C. Injector temperature was 225 °C with transfer line temperature of 285 °C. Metalaxyl was confirmed with retention time of 16.2 min. The limit of quantification was 0.007 $\mu g\,mL^{-1}$ and the limit of detection was $2.54\times 10^{-4}\,\mu g\,mL^{-1}.$

2.7. Trifluralin extraction

The same sample preparation, column preparation, sample elution, and analysis protocols were used as for metalaxyl except as noted. Initial temperature was 115 °C, increasing 30 °C min⁻¹ to 280 °C, with a 6 min hold at 280 °C. Injector temperature was 260 °C with transfer line temperature of 285 °C. The limit of quantification was 0.014 μ g mL⁻¹ and the limit of detection was 0.004 μ g mL⁻¹.

2.8. Nitrate analysis

Concentration of nitrate–nitrogen (NO_3^--N) was determined by a segmented flow analysis system (O-I-Analytical Flow Solution IV analyzer, O-I-Analytical, College Station, TX, United States) to measure NO_3^--N plus nitrite–nitrogen (NO_2-N) . Nitrate–nitrogen in the sample was reduced quantitatively to NO_2-N by cadmium reduction and along with any NO₂–N present in the sample, diazotized with sulfanilamide and coupled with *N*-1-naphthylethylenediamine dihydrochloride. Total NO₂–N was then detected colorimetrically as a colored azo dye at 540 nm. Nitrite–nitrogen quantity present in the original sample was measured without the cadmium reduction. Nitrate–nitrogen was quantified using WinFLOWTM software to subtract NO₂–N present in the original sample from NO₂–N in the original sample plus NO₂–N formed from the cadmium reduction of NO₃⁻–N. The method detection limit for this method is 0.004 mg L⁻¹ NO₃⁻–N plus NO₂–N. For details on reagents used, system calibration, and sample preparation, readers are directed to O-I-Analytical (2001).

2.9. Statistical analysis

Data from each trifluralin or metalaxyl application were analyzed separately. Comparisons among treatments were made for trifluralin, metalaxyl, and NO₃⁻–N concentration and quantity in water leaving PAs. Trifluralin and metalaxyl data following each application were analyzed as repeated measures using PROC MIXED procedure of SAS (SAS version 9.1; SAS Institute, Cary, NC, USA). When treatment main effects or treatment by day interactions were significant at the 0.05 level, treatment means were separated by day using a *t*-test in the PDIFF option of the LSMEANS statement and the SLICE option of PROC MIXED ($p \le 0.05$). Nitrate–nitrogen data were subjected to ANOVA using PROC GLM procedure of SAS and when significant ($p \le 0.05$), treatment means were separated by Duncan's multiple range test and were declared significant at $p \le 0.05$.

A second analysis (Analysis Two) was conducted to evaluate the effectiveness of PAs on the remediation of NB runoff. Data from all PAs (with and without plants) were pooled to compare runoff leaving NBs to water moving out of PAs. In this analysis, there were twelve replications with each NB and the associated PA serving as a treatment replicate. Each chemical application was analyzed separately. Statistical analysis was performed on chemical concentration and quantity as repeated measures for metalaxyl and trifluralin and ANOVA for NO₃⁻–N data as previously described.

3. Results and discussion

3.1. Analysis one: phytoremediation by salix and sambucus grown in phytoremediation areas

Initial and final plant height and width are shown in Table 2. Both *Salix* and *Sambucus* plants were considered marketable by the end of the experiment based upon plant size and subjective visual inspection of plant quality. The presence of container-grown *Salix* or *Sambucus* plants in PAs did not reduce trifluralin or metalaxyl concentrations greater than control PAs. There was no treatment effect on trifluralin (2004) or metalaxyl (2006) concentration in water leaving PAs on any collection day (p > 0.05; data not presented). In 2005, on the day of the second trifluralin application, the concentration of trifluralin in water leaving *Salix* PAs [0.044 mgL^{-1} (mean) $\pm 0.026 \text{ mgL}^{-1}$ (SE)] was higher than *Sambucus* PAs ($0.006 \text{ mgL}^{-1} \pm 0.002 \text{ mgL}^{-1}$; $p \le 0.05$); however, the control treatment ($0.011 \text{ mgL}^{-1} \pm 0.004 \text{ mgL}^{-1}$) was not different from the *Salix* or *Sambucus* treatments (data not presented). There were no other differences in 2005.

For trifluralin (2004 or 2005) and metalaxyl (2006) quantity in water leaving PAs there were no treatment effects on 8 of 10 collection days, 11 of 12 collection days, and 14 of 15 collection days, respectively (p > 0.05). In 2004, for the third trifluralin application on 0 DAA (the day of the third trifluralin application)

Table	2
-------	---

Initial and final height and width of Salix alba 'Britzensis' and Sambucus nigra 'Aurea' plan	ts grown in phytoremediation areas.

Year	Plant growth parameters	Plant growth parameters ^a (cm)				
Taxa	Initial height	Final height	Initial width	Final width		
2004	2-June		2-June			
Salix alba 'Britzensis'	44 ± 1.0	N/A ^b	45 ± 0.7	N/A		
Sambucus nigra'Aurea'	51 ± 1.1	N/A	50 ± 1.3	N/A		
2005	16-May	15-Jul	16-May	15-Jul		
Salix alba 'Britzensis'	58 ± 0.0	160 ± 1.9	63 ± 0.8	102 ± 1.8		
'Aurea'	55 ± 0.0	91 ± 1.9	66 ± 1.1	90 ± 2.2		
2006	17-Jul	25-Aug	17-Jul	25-Aug		
Salix alba 'Britzensis'	121 ± 2.5	133 ± 3.6	110 ± 3.6	106 ± 3.1		
Sambucus nigra'Aurea'	71 ± 0.9	78 ± 0.9	73 ± 1.0	87 ± 1.9		

^a Plant height was measured from the top of the container rim to the highest terminal bud. Plant height or width ± standard error of the mean of 80 plants of each species on each date. On 16 May 2005, standard errors of 0.0 correspond to pruning of *Salix* and *Sambucus* to 58 or 55 cm at the beginning of the experiment. Shorter plant heights and widths from 15 July 2005 to 17 July 2006 resulted from pruning before the experiment began in May.

^b Data not available.

and 1 DAA, trifluralin quantity leaving *Salix* PAs $(4.18 \pm 0.98 \text{ mg} \text{ and } 3.20 \pm 0.76 \text{ mg})$ was greater than trifluralin quantities leaving *Sambucus* PAs $(1.67 \pm 0.33 \text{ mg} \text{ and } 1.49 \pm 0.57 \text{ mg})$ and control PAs $(2.19 \pm 0.36 \text{ mg} \text{ and } 1.42 \pm \text{SE} 0.27 \text{ mg}, p \le 0.05)$. Similarly in 2005, on the day of the third trifluralin application, trifluralin quantities leaving *Salix* PAs $(1.30 \pm 0.49 \text{ mg})$ were greater than quantities leaving *Sambucus* $(0.376 \pm 0.22 \text{ mg})$ and control $(0.15 \pm 0.08 \text{ mg})$ PAs. On the day of the first metalaxyl application, water leaving *Sambucus* PAs contained larger quantities of metalaxyl ($36.20 \pm 22.41 \text{ mg}$) compared to *Salix* PAs $(2.41 \pm 2.41 \text{ mg})$ and control PAs $(1.00 \pm 0.35 \text{ mg})$.

Phytoremediation area treatment did not affect NO₃⁻-N concentration in water leaving PAs on 8 of 9 collection days or quantity on all 9 collection days, respectively (p > 0.05; data not presented). On 30 May 2004, nitrate concentration in water leaving Sambucus PAs $(2.824 \text{ mg L}^{-1} \pm 0.246 \text{ mg L}^{-1})$ was greater than that leaving control PAs $(1.656 \text{ mg L}^{-1} \pm 0.423 \text{ mg L}^{-1})$, but nitrate concentration leaving Salix PAs (1.911 mg $L^{-1} \pm 0.138$ mg L^{-1}) was not different from that of *Sambucus* or control PAs (p < 0.05). Leverenz et al. (2010) reported similar results that during the first 5 months planted and unplanted woodchip wetlands removed an average of 99.7% of the influent nitrate. However, there were no differences in effluent nitrate concentrations between wetlands planted with T. latifolia and unplanted wetlands in 2008, or between planted and unplanted wetlands during the first 4 months of the 2007 study (Leverenz et al., 2010). Wang and Duggin (2008) reported that there was no difference in NO₃⁻-N removal between trees and pasture, and among tree treatments. However, the tree treatments (two species used: Eucalyptus camaldulensis Dehnh. and Casuarina cunninnghamiana Mq.) in a vegetation soil system retained 36.3% and 37.6% more NO_3^- – N than bare soil for the A–B horizon interface flow and the B horizon flow, respectively. Although the trees in the study by Wang and Duggin (2008) were not grown in containers and therefore cannot be directly compared to our study it does show that trees can be valuable components in a vegetated filter strip for NO₃⁻–N removal.

We initially postulated that PAs with container-grown plants would significantly reduce chemicals leaving PAs through plant and media uptake compared to control PAs given results of previous studies. Elsaesser et al. (2011), using the toxic units approach, calculated pesticide toxicity was reduced 95% in two vegetated wetland cells and 79% in non-vegetated cells, and mean reduction of pesticide peak concentration was 91% in vegetated wetland cells and 72% in non-vegetated wetland cells. Stearman et al. (2003) reported that during a two year period, constructed wetland cells vegetated with *Scirpus vilidus* removed 82.4% and 77.1% of the herbicides metolachlor and simazine compared with 63.2% and 64.3% removal by wetland cells without plants. Wilson et al. (2000) reported that after 3 days of growing in a nutrient solution containing metalaxyl, plant tissues of T. latifolia had significant accumulations of radiolabeled metalaxyl primarily in the leaves. Metalaxyl concentration in the nutrient solution after 7 days had decreased by 34%, and nearly all of the radiolabeled metalaxyl removed from the nutrient solution was detected in the plants. Li et al. (2002) showed that trifluralin uptake, metabolism, and the formation of bound residues occurred in L. multiflorum growing in solutions containing trifluralin. However, in the current study there was no evidence of significant metalaxyl or trifluralin phytoremediation by container-grown Salix or Sambucus plants in PAs compared to control PAs without plants. In contrast to the closed systems used by Wilson et al. (2000) and Li et al. (2002), in which plant roots were suspended in a chemical containing solution, our system was an open, free-flowing system. In the current study, Salix and Sambucus roots were confined to the volume of the container with access to water from the container substrate via five drainage holes in the bottom of each container. However, it is unlikely that container substrate or drainage holes restricted root access to chemicals and nitrates in the water in PAs in our study. Plants growing in PAs did not wilt at any time during the study and no supplemental irrigation was added from the top of the container, except naturally occurring precipitation, indicating that water and the chemicals in the water in PAs were readily available to roots in container substrate. A limitation of the current study is that a plant tissue analysis was not performed; therefore plant uptake of metalaxyl or trifluralin could not be confirmed.

3.1.1. Analysis two: phytoremediation of nursery runoff by phytoremediation areas

Because the presence of container-grown plants in PAs did not increase phytoremediation of metalaxyl, trifluralin, or NO_3^- –N; we suspect that the remediation which occurred was related to other remediation processes in addition to plant uptake. Even though phytoremediation areas containing container-grown plants held approximately 290L compared to 440L of water in PAs without plants, consistent differences in chemical concentration or quantity did not occur in PAs with and without plants. Therefore, data were pooled to investigate the effect of channeling runoff through PAs, and water leaving all 12 PAs (PAs with and without plants) was compared to runoff collected from NBs.

Channeling runoff through PAs reduced trifluralin concentrations and quantity on 10 of 11 collection days (2004) and on 11 of 12 collection days (2005; Fig. 2A–D). On the day of application for applications 1, 2, and 3 in 2004, the PAs reduced trifluralin concentrations in runoff by 80% (from 0.129 ± 0.025 mg L⁻¹

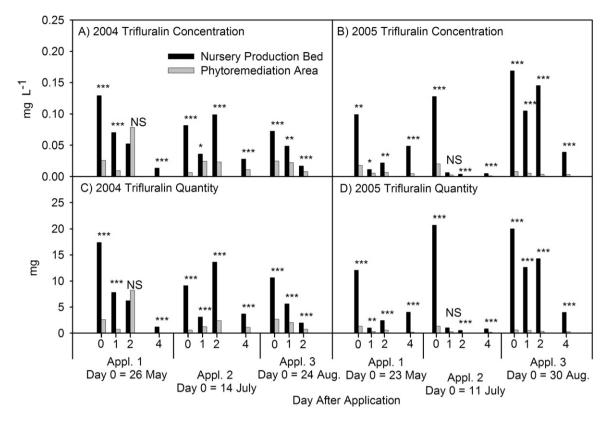


Fig. 2. Trifluralin concentration and quantity measured from nursery beds (NB) and water exiting phytoremediation areas that nursery runoff was channeled through. Three applications of trifluralin were made in 2004 and 2005 with measurements taken on the day of application (day 0) and at 1, 2, and 4 days after application (DAA). Trifluralin was applied at 6.25 g active ingredient (ai) per NB (3.37 kg ai ha⁻¹) at each application. In 2004, on 4 DAA and for application 1, there was no trifluralin detected leaving production areas. No samples were collected on 4 DAA for application 3 due to heavy rain. For each application when treatment main effect or treatment by day interaction were significant (*p* < 0.05) treatment means were sliced by day and means separated using a *t*-test (^{NS}, *, **, *** Nonsignificant or significant at *p* < 0.05, 0.01, or 0.001, respectively). *n* = 12.

to $0.026 \pm 0.008 \text{ mg L}^{-1}$), 92% (from $0.082 \pm 0.011 \text{ mg L}^{-1}$ to $0.006 \pm 0.003 \text{ mg L}^{-1}$), and 66% (from $0.073 \pm 0.009 \text{ mg L}^{-1}$ to $0.025 \pm 0.004 \text{ mg L}^{-1}$), respectively (Fig. 2A). Similarly, on the day of application for applications 1, 2, and 3 in 2005, PAs reduced trifluralin concentrations in NB runoff by 82% (from $0.100 \pm 0.023 \text{ mg L}^{-1}$ to $0.018 \pm 0.005 \text{ mg L}^{-1}$), 84% (from $0.128\pm 0.022\,mg\,L^{-1}$ to $0.020\pm 0.009\,mg\,L^{-1}),$ and 95% (from $0.169 \pm 0.038 \text{ mg L}^{-1}$ to $0.008 \pm 0.003 \text{ mg L}^{-1}$; Fig. 2B). Overall average trifluralin concentrations from water leaving PAs were 64% $(0.021 \pm 0.003 \text{ mg L}^{-1})$ and 90% $(0.007 \pm 0.001 \text{ mg L}^{-1})$ lower than trifluralin concentrations in NB runoff in 2004 $(0.059 \pm 0.005 \text{ mg L}^{-1})$ and $2005 (0.065 \pm 0.007 \text{ mg L}^{-1})$. Overall average trifluralin quantities leaving PAs were reduced by 72% (from $7.31 \pm 0.67 \text{ mg}$ to $2.03 \pm 0.29 \text{ mg}$) in 2004 compared to runoff from NBs and reduced by 94% (from 7.81 ± 0.86 mg to 0.51 ± 0.07 mg) in 2005 compared to runoff from NBs.

Total quantity of trifluralin captured in NB runoff during the period of analysis following each application in 2004 and 2005 was <1% of that applied. Total quantity of trifluralin captured in this experiment was similar to that reported by Wilson et al. (1996) where 0.3% and 1.1% of applied trifluralin moved in nursery runoff during the first 5 days after treatment and 0.9% and 0.3% of applied trifluralin was recovered from nursery runoff in clay/gravel and grass waterways (Brigg et al., 1999). Wilson et al. (1995) reported 1%, 4%, and 4% of applied granular trifluralin was detected in runoff from gravel, plastic, and fabric ground covers, respectively, during a 30-day period following application of 2.2 kg active ingredient ha⁻¹.

Trifluralin is subject to degradation by soil microorganisms, UV light (Leitis and Crosby, 1974), and volatilization (Extension Toxicology Network, 1993; Wheeler et al., 1979); and these processes likely contributed to the remediation of trifluralin in PAs. Dimou et al. (2004) reported that as dissolved organic matter (DOM) in natural waters increased, trifluralin photolysis decreased. Although microorganisms and sediment in PA water may have reduced trifluralin photolysis by absorbing and scattering light, these can also be mechanisms to remediate trifluralin. Trifluralin is strongly adsorbed on soils and not highly water soluble. Therefore, it was likely that trifluralin entering PAs that came in contact with sediments or container substrate was bound and, although no longer available for photolysis, did not move out of PAs. Dimou et al. (2004) reported that trifluralin photolysis was increased by a reaction with hydroxyl radicals produced from NO₃⁻-N photolysis because the rate of trifluralin degradation increased by up to 63% when $10 \text{ mg L}^{-1} \text{ NO}_3^{-}-\text{N}$ was in the water solution. Although NO₃⁻-N concentrations in our experiment did not reach the 10 mg L^{-1} concentration reported by Dimou et al. (2004), we hypothesize that NO₃⁻-N presence contributed to trifluralin photolysis and removal in PAs.

Metalaxyl concentration was lower in water leaving PAs compared to NB runoff on 2 of 15 collection days (Fig. 3A). On the day of application for applications 1 and 3, metalaxyl concentrations were reduced by 90% (from $2.189 \pm 0.937 \text{ mgL}^{-1}$ to $0.231 \pm 0.080 \text{ mgL}^{-1}$) and 63% (from $2.210 \pm 0.453 \text{ mgL}^{-1}$ to $0.812 \pm 0.304 \text{ mgL}^{-1}$) as NB runoff was channeled through PAs (Fig. 3A). The overall average metalaxyl concentration in water leaving PAs was 62% lower (reduced from $0.576 \pm 0.119 \text{ mgL}^{-1}$ to $0.217 \pm 0.031 \text{ mgL}^{-1}$) than in NB runoff. Dunn et al. (2011) reported similar percent reductions in metalaxyl concentrations in overland flow through buffer zones of 34% and 88% at distances of 10 m and

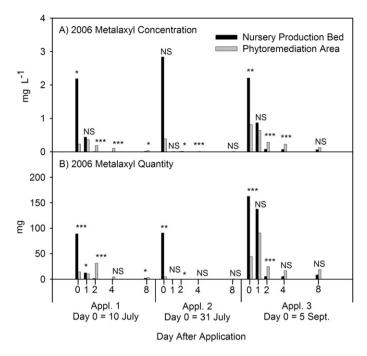


Fig. 3. Metalaxyl concentration and quantity measured from 3 m × 6 m nursery beds (NB) and water exiting phytoremediation areas that nursery runoff was channeled through. Three applications of metalaxyl were made in 2006 with measurements taken on the day of application (day 0) and at 1, 2, 4, and 8 days after application (DAA). For each application metalaxyl was applied at 0.646 g active ingredient (ai) per NB (0.347 kg ai ha⁻¹). For each application when treatment means were sliced by day interaction were significant (p < 0.05) treatment means were sliced by p < 0.05, 0.01, or 0.001, respectively). n = 12.

30 m when compared to concentrations at the field's edge (0 m). However these reductions were not significant at the 0.05 probability level.

The quantity of metalaxyl in PAs was lower than the amount in NB runoff on 4 of 15 collection days, with differences occurring on the day of application for all 3 application days and 1 DAA for application 1 (Fig. 3B). Channeling water through PAs reduced the overall average metalaxyl quantity leaving NBs by 48% (from 34.32 ± 7.98 mg to 17.69 ± 2.78 mg). On the day of application for applications 1, 2, and 3; 97.48 ± 30.40 mg, 90.78 ± 37.94 mg, and 162.66 ± 44.25 mg, respectively, of the applied 646 mg of metalaxyl per NB per application was recovered in runoff from NBs (Fig. 3B).

The large quantity of metalaxyl moving in runoff on the day of application can be explained by the high water solubility of metalaxyl, which resulted in high losses from NBs on the day of application. The majority of metalaxyl moved from NBs into PAs on the day of application resulting in an initial buildup of metalaxyl in PAs, with smaller quantities lost in runoff on the remaining sample days. Although the initial loss of metalaxyl from NBs was diluted by water in PAs, the initial quantity along with additional metalaxyl moving into PAs was large enough to equal or exceed metalaxyl losses from NBs on subsequent DAA. This explains the few differences in metalaxyl concentration and quantity that occurred following the day of application and why metalaxyl concentrations and quantities in PAs were higher than NB runoff when differences did occur.

Unlike trifluralin, metalaxyl is highly water soluble (Toxicology Data Network, 2003) and more prone to movement in runoff as was evident in the concentrations and quantities captured on the days of application in this experiment. Similar to trifluralin, metalaxyl is subject to degradation in soil by microorganisms (Droby

Table 3

Nitrate concentration and quantity measured from $3 \text{ m} \times 6 \text{ m}$ nursery beds (NB) and water exiting phytoremediation areas (PA) that nursery runoff was channeled through. Nitrates were analyzed for day 4 after application of trifluralin in 2004 and 2005 and metalaxyl in 2006. In 2004 and 2005, total applied N was 224.06 g per NB (121 kg ha⁻¹). In 2006, total applied N was 291.72 g per NB (157 kg ha⁻¹).

Year	Date	Location	Nitrate (r	Nitrate (mg L ⁻¹)		Nitrate (mg)	
2004	30 May	NB	2.137	a ^A	157	a	
		PA	2.130	a	157	a	
	18 Jul	NB	1.288	b	172	a	
		PA	2.269	a	226	a	
	26 Aug	NB	0.984	a	111	a	
		PA	2.132	a	214	a	
2005	27 May	NB	9.116	a	584	a	
		PA	4.327	b	356	a	
	15 Jul	NB	2.139	a	315	a	
		PA	0.917	b	160	b	
	3 Sep	NB	0.604	a	054	a	
		PA	0.283	b	027	a	
2006	14 Jul	NB	6.771	a	373	a	
		PA	0.235	b	006	b	
	4 Aug	NB	8.322	a	627	a	
		PA	2.583	b	250	b	
	9 Sep	NB	1.470	a	169	a	
		PA	0.941	b	090	b	

^A Means within each column for each day with the same letter are not significantly different ($p \le 0.05$). Means separated by Duncan's multiple range test. n = 12.

and Coffey, 1991; Huaguo et al., 1995). Sukul and Spiteller (2001) reported that after 60 days, 5.3–14.7% degradation of metalaxyl in sterilized soil was due to abiotic factors other than light and that 35.8–57.3% metalaxyl degradation in non-sterilized soil was due to microbes. Photodegradation of metalaxyl in soils and aqueous solutions have been reported at various rates by Sharom and Edgington (1982), Sukul et al. (1992), and Yao et al. (1989). Plant uptake, runoff dilution, degradation by microorganisms in PAs, adsorption to container substrates, and photolysis were mechanisms that likely contributed to reductions in metalaxyl as runoff moved through PAs.

Pyrethroids were found to be overwhelmingly associated with suspended solids in constructed wetlands with sedimentation the primary mechanism for the high pesticide removal efficiency and most pyrethroids and chlorpyrifos were moderately persistent in wetland sediments under flooded, anaerobic conditions but significantly persistent under dry, aerobic conditions (Budd et al., 2011). Although no pyrethroids were evaluated in this study, metalaxyl adsorption to soils has been reported by Sukul and Spiteller (2001). During periods when irrigation is not applied the drains in PAs could be closed to keep PAs flooded and anaerobic. This would help keep pesticides bound in sediments, such as pyrethroids, from becoming more highly persistent under aerobic conditions with the potential to accumulate over time and be discharged during a heavy rain event. Furthermore, the PAs used in this experiment can be cleaned periodically and accumulated sediment removed to reduce the potential for long term buildup of sediment bound pesticides.

3.1.2. NO_3^- –N phytoremediation

Phytoremediation areas reduced NO₃⁻–N concentration and quantity moving in runoff on 7 of 9 days and 4 of 9 days sampled during the 3 growing seasons of the experiment (Table 3). In 2004 there were no differences in NO₃⁻–N concentration or quantity lost from PAs compared to losses from NBs except on 18 July. In 2005 NO₃⁻–N concentration in water leaving PAs was lower than NB NO₃⁻–N concentration on the three sample dates. Nitrate–nitrogen quantity lost from PAs (584 ± 189 mg) in 2005 was 49% lower than NO₃⁻–N in NB runoff (356 ± 79 mg) on 15 July with no differences on 27 May or 3 September. On each NO₃⁻–N collection day in

2006, NO₃⁻–N concentration and quantity in water leaving PAs was lower compared to NB runoff. Phytoremediation areas reduced the NO₃⁻-N quantity in runoff from NBs by 98% (373–6 mg), 60% (627 mg to 250 mg), and 47% (169–90 mg) on 14 July, 4 Aug, and 9 Sept, respectively. Dunn et al. (2011) reported 38% (significant at the 0.05 probability level) and 84% (not significant at the 0.05 probability level) reduction of NO₃⁻-N in overland flow in buffer zones at 10 m and 30 m from the fields edge (0 m) compared to concentrations at the field's edge. Nitrate-nitrogen in runoff water channeled through PAs with container-grown plants was likely absorbed by plants growing in PAs. Nitrate-nitrogen in aqueous solution is also subject to photolysis (Mack and Bolton, 1999). Nitrate-nitrogen could also have been removed through denitrification by microorganisms in PAs (Reeves, 1972). A combination of these processes likely contributed to NO₃⁻-N reductions as runoff was channeled through PAs.

Reductions in trifluralin, metalaxyl, and NO₃⁻-N in water leaving PAs compared to runoff from NBs likely resulted from a combination of the processes described above as well as dilution by water in PAs. The amount of dilution would depend on a number of factors including: volume of runoff entering each PA, rate at which runoff entered PA, rate of water draining out of PA, level of water in PA prior to irrigation event (losses from evaporation and plant transpiration), and presence of plants or no plants in the PA. The average runoff volume collected on each sample day was 106 L per NB. If the water level in PAs was just below the bottom of the drainage pipe, runoff volume entering PAs would have displaced approximately the same volume of water leaving the PA through the drain on the opposite side. Phytoremediation areas also slowed down the movement of runoff into the environment, allowing time for other processes involved in phytoremediation to occur. Pesticide removal from container production sites in gravel subsurface flow constructed wetlands was greatest at a lower flow rate in vegetated cells compared with higher flow rates and non-vegetated cells (Stearman et al., 2003).

4. Conclusions

Remediation of trifluralin, metalaxyl, and NO_3^--N by containergrown *S. alba 'Britzensis'* and *S. nigra* 'Aurea' in PAs was not greater than remediation in PAs without plants. However, channeling NB runoff through PAs with or without semi-aquatic plants was highly effective in remediating nursery runoff from NBs treated with trifluralin and metalaxyl.

Even though metalaxyl is highly water soluble, concentrations recovered from NBs and PAs did not exceed the LC_{50} (51.5 mg L^{-1} under a 48 h exposure duration) for D. magna (water flea) or the LC_{50} (130 mg L^{-1} under a 96 h exposure duration) for O. mykiss (rainbow trout; Fig. 3A; USEPA, 2007). Similarly, trifluralin concentrations in runoff from NBs did not exceed the 48 h exposure LC₅₀ of 0.560 mg L⁻¹ for *D. magna* (water flea; U.S. Department of Interior, Fish and Wildlife Service, 1980). Channeling NB runoff through PAs reduced trifluralin concentrations from levels that exceeded the LC_{50} (0.041 mg L⁻¹ for a 96 h exposure duration) for *O. mykiss* on 7 of 11 days in 2004 and 6 of 12 days in 2005 to levels below the LC_{50} on 10 of 11 days in 2004 and all 12 days in 2005 (Fig. 2A and B; U.S. Department of Interior, Fish and Wildlife Service, 1980). Following the third trifluralin application in 2005, trifluralin concentrations in NB runoff exceeded the 96 h LC_{50} of 0.041 mg L^{-1} for 0. mykiss until day 4 when concentrations were 0.039 mg $L^{-1} \pm 0.006$ mg L^{-1} , with the LC₅₀ still within the variability of the data (Fig. 2B). However, trifluralin concentrations leaving PAs during this time were below the LC₅₀ for O. mykiss (Fig. 2B). Nitrate-nitrogen levels in NB runoff on 27 May 2005 and 4 August 2006 were reduced

from $9.116 \pm 0.006 \text{ mg L}^{-1}$ and $8.332 \pm 0.583 \text{ mg L}^{-1}$, close to the 10 mg L^{-1} federal drinking water standard, to $4.327 \pm 0.769 \text{ mg L}^{-1}$ and $2.583 \pm 0.697 \text{ mg L}^{-1}$ (Table 3; USEPA, 2009). In areas where aquatic organisms and human drinking water supplies are located the use of PAs would reduce environmental contamination and possible harmful effects to aquatic organisms from runoff containing trifluralin or NO₃⁻–N.

Effectiveness of PAs in reducing trifluralin, metalaxyl, and NO₃⁻-N in this experiment likely resulted from a number of processes including: dilution by water in the PA, plant uptake, degradation by microorganisms in PAs, adsorption to organic container substrates, and PA sediments, photolysis, and volatilization. In addition to reducing nutrients and pesticide losses, PAs including container-grown S. alba 'Britzensis' and S. nigra 'Aurea' as components of the PA provide the additional benefit of utilizing the land area occupied by PAs for container plant production. Phytoremediation areas would be easier to clean than constructed wetlands and would allow for accumulated sediment to be removed in order to prevent potential long-term buildup of sediment bound pesticides in PAs. Future research should target the evaluation of additional commercially produced species that could be grown in PAs for runoff phytoremediation and commercial sale, and the various mechanisms of nutrient and pesticide removal from runoff in PAs.

Acknowledgements

Funding for our research was provided by Michigan State University Project GREEEN and the Horticulture Research Institute. Additional support was provided by Wilbro, Inc.; Spring Meadow Nursery, Inc.; Zelenka Nursery, Inc. (now Berry Family of Nurseries LLC); Walters Gardens, Inc.; Renewed Earth LLC; and Harrell's LLC. We would also like to gratefully acknowledge those who worked on this project in the field and laboratory.

References

- Asmussen, L.E., White Jr., A.W., Hauser, E.W., Sheridan, J.M., 1977. Reduction of 2,4-D load in surface runoff down a grassed waterway. J. Environ. Qual. 6, 159–162.
- Baz, M., Fernandez, R.T., 2002. Evaluating woody ornamentals for use in herbicide phytoremediation. J. Am. Soc. Hort. Sci. 127, 991–997.
- Beeson Jr., R.C., 2006. Relationship of plant growth and actual evapotranspiration to irrigation frequency based on management allowed deficits for container nursery stock. J. Am. Soc. Hort. Sci. 131 (1), 140–148.
- Bhandary, R., Whitwell, T., Briggs, J., 1997a. Growth of containerized landscape plants is influenced by herbicides residues in irrigation water. Weed Technol. 11, 793–797.
- Bhandary, R., Whitwell, T., Briggs, J., Fernandez, R.T., 1997b. Influence of surflan (oryzalin) concentrations in irrigation water on growth and physiological processes of *Gardenia jasminoides* radicans and *Pennisetum rupelli*. J. Environ. Hort. 15, 169–172.
- Briggs, J.A., Riley, M.B., Whitwell, T., 1998. Quantification and remediation of pesticides in runoff water from containerized plant production. J. Environ. Qual. 27, 814–820.
- Brigg, J.A., Whitwell, T., Riley, M.B., 1999. Remediation of herbicides in runoff water from container plant nurseries utilizing grassed waterways. Weed Technol. 13 (1), 157–164.
- Briggs, J.A., Whitwell, T., Fernandez, R.T., Riley, M.B., 2002a. Formulation effects on isoxaben and trifluralin in runoff water from container plant nurseries. Weed Sci. 50, 536–541.
- Briggs, J.A., Whitwell, T., Fernandez, R.T., Riley, M.B., 2002b. Effect of integrated pest management strategies on chlorothalonil, metalaxyl and thiophanate-methyl runoff at a container nursery. J. Am. Soc. Hort. Sci. 127, 1018–1024.
- Budd, R., O'geen, A., Goh, K.S., Bondarenko, S., Gan, J., 2011. Removal mechanisms and fate of insecticides in constructed wetlands. Chemosphere 83, 1581–1587.
- Businelli, M., Patumi, M., Marucchini, C., 1984. Identification and determination of some metalaxyl degradation products in lettuce and sunflower. J. Agric. Food Chem. 32, 644–647.
- Camargo, J.A., Alonso, A., Salamanca, A., 2005. Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. Chemosphere 58, 1255–1267.
- Camper, N.D., Whitwell, T., Keese, R.J., Riley, M.B., 1994. Herbicide levels in nursery containment pond water and sediments. J. Environ. Hort. 12, 8–12.

Cohen, Y., Coffey, M.D., 1986. Systemic fungicides and the control of oomycetes. Ann. Rev. Phytopathol. 24, 311–338.

- Cole, D.J., Owen, W.J., 1987. Metabolism of metalaxyl in cell suspension cultures of Lactuca sativa L. and Vitis vinifera L. Pestic. Biochem. Physiol. 28, 354–361.
- Cunningham, S.D., Berti, W.R., 1993. Remediation of contaminated soils with green plants: an overview. In Vitro Cell. Dev. Biol. 29, 207–212.
- Dillaha III, T.A., Sherrard, J.H., Lee, D., 1989. Long term effectiveness of vegetated filter strips. Water Environ. Technol. 1, 418-421.
- Dimou, A.D., Sakkas, V.A., Albanis, T.A., 2004. Trifluralin photolysis in natural waters and under the presence of isolated organic matter and nitrate ions: kinetics and photoproduct analysis. J. Photochem. Photobiol. A: Chem. 163, 473–480.
- Dirr Michael, A., 2009. Manual of Woody Landscape Plants, 6th ed. Stipes Publishing L.L.C, Champaign, IL 61824.
- Droby, S., Coffey, M.D., 1991. Biodegradation process and the nature of metabolism of metalaxyl in soil. Ann. Appl. Biol. 118, 543–553.
- Dunn, A.M., Julien, G., Ernst, W.R., Cook, A., Doe, K.G., Jackman, P.M., 2011. Evaluation of buffer zone effectiveness in mitigating the risks associated with agricultural runoff in Prince Edward Island. Sci. Total Environ. 409, 868–882.
- Elsaesser, D., Blankenburg, A.B., Geist, A., Maehlum, T., Schulz, R., 2011. Assessing the influence of vegetation on reduction of pesticide concentration in experimental surface flow constructed wetlands: application of the toxic units approach. Ecol. Eng. 37, 955–962.
- Extension Toxicology Network, 1993. Pesticide Information Profile: Trifluralin. Project of Cooperative Extension Offices of Cornell University, Michigan State University, Oregon State University, and University of California at Davis. Website: http://pmep.cce.cornell.edu/profiles/extoxnet/pyrethrinsziram/trifluralin-ext.html (accessed 03.04.09).
- Fare, D.C., Gilliam, C.H., Keever, G.J., Olive, J.W., 1994. Cyclic irrigation reduces container nitrate–nitrogen concentration. HortScience 29 (12), 1514–1517.
- Fare, D.C., Gilliam, C.G., Keever, G.J., 1992. Monitoring irrigation at container nurseries. HortTechnology 2, 75–78.
- Fernandez, R.T., Whitwell, T., Riley, M.B., Bernard, C.R., 1999. Evaluating semiaquatic herbaceous perennials for use in herbicide phytoremediation. J. Am. Soc. Hort. Sci. 124 (5), 539–544.
- Field, J.A., Thurman, E.M., 1996. Glutathione conjunction and containment transformation. Environ. Sci. Technol. 30, 1413–1418.
- Gilliam, C.H., Fare, D.C., Beasley, A., 1992. Nontarget herbicide losses from application of granular Ronstar to container nurseries. J. Environ. Hort. 10 (3), 175–176.
- Hoagland, D.R., Arnon, D.I., 1938. The Water Culture Method for Growing Plants Without Soil. Circ. 347. Univ. of California Agric. Exp. Stn., Berkley, CA, United States.
- Huaguo, W., Peng, G., Qi, M., 1995. Study on the residues and degradation of ¹⁴Cmetalaxyl in soils. Acta Agric. Univ. Pekin. 21, 395–401.
- Kimmel, E.C., Casida, J.E., Ruzo, L.O., 1986. Formamidine insecticides and chloroacetanilide herbicides: disubstituted anilines and nitrobenzenes as mammalian metabolites and bacterial mutagents. J. Agric. Food Chem. 34, 157–161.
- Komossa, D., Langbartels, C., Sandermann, H.J., 1995. Metabolic processes for organic chemicals in plants. In: Trapp, S., McFarlane, J.C. (Eds.), Plant Contamination: Modeling and Simulation of Organic Chemical Processes. CRC Press, Boca Raton, FL, pp. 69–106.
- Kuzovkina, Y.A., Volk, T.A., 2009. The characterization of willow (*Salix* L.) varieties for use in ecological engineering applications: co-ordination of structure, function, and autecology. Ecol. Eng. 35, 1178–1189.
- Leitis, E., Crosby, D.G., 1974. Photodecomposition of trifluralin. J. Agric. Food Chem. 22, 842–848.
- Leverenz, H.L., Haunschild, K., Hopes, G., Tchobanoglous, G., Darby, J.L., 2010. Anoxic treatment wetlands for denitrification. Ecol. Eng. 36, 1544–1551.
- Li, H., Sheng, G., Sheng, W., Xu, O., 2002. Uptake of trifluralin and lindane from water by ryegrass. Chemosphere 48, 335–341.
- Mack, J., Bolton, J.R., 1999. Photochemistry of nitrite and nitrate in aqueous solution: a review. J. Photochem. Photobiol. A: Chem. 128, 1–13.
- Magette, W.L., Brinsfield, R.B., Palmer, R.E., Wood, J.D., 1989. Nutrient and sediment removal by vegetated filter strips. Trans. ASAE 32, 663–667.
- Maillard, E., Payraudeau, S., Faivre, E., Grégoire, C., Gangloff, S., Imfeld, G., 2011. Removal of pesticide mixtures in a stormwater wetland collecting runoff from a vineyard catchment. Sci. Total Environ. 409, 2317–2324.
- Mickelson, S.K., Baker, J.L., 1993. Buffer Strips for Controlling Herbicide Runoff Losses. Paper no. 93-2084. ASAE, St. Joseph, MI.
- O-I-Analytical, 2001. Nitrite Plus Nitrite Nitrogen and Nitrite Nitrogen in Soil and Plant Extracts by Segmented Flow Analysis (SFA). Publication No. 15300301, College Station, TX, 27 pp.
- Owen, W.J., Donzel, B., 1986. Oxidative degradation of chlortoluron, propiconazole, and metalaxyl in suspension of cultures of various crop plants. Pestic. Biochem. Physiol. 26, 75–89.
- Raskin, I., Kumar, P.B.A.N., Dushenkov, S., Salt, D.E., 1994. Bioconcentration of heavy metals by plants. Curr. Opin. Biotechnol. 5, 285–290.

- Reeves, T.G., 1972. Nitrogen removal: a literature review. J. Water Pollut. Control Fed. 44 (10), 1895–1908.
- Riley, M.B., Keese, R.J., Camper, N.D., Whitwell, T., Wilson, P.C., 1994. Pendimethalin and oxyfluorfen residues in pond water and sediment from container plant nurseries. Weed Technol. 8, 299–303.
- Sharom, M.S., Edgington, L.V., 1982. The adsorption, mobility, and persistence of metalaxyl in soil and aqueous system. Can. J. Plant Pathol. 4, 333–340.
- Skimina, C.A., 1992. Recycling water, nutrients, and waste in the nursery industry. HortScience 27, 968–971.
- Stearman, G.K., George, D.B., Carlson, K., Lansford, S., 2003. Pesticide removal from container nursery runoff in constructed wetland cells. J. Environ. Qual. 32, 1548–1556.
- Stehle, S., Elsaesser, D., Gregoire, C., Imfeld, G., Niehaus, E., Passeport, E., Payraudeau, S., Schäfer, R.B., Tournebize, J., Schulz, R., 2011. Pesticide risk mitigation by vegetated treatment systems: a meta-analysis. J. Environ. Qual. 40, 1068–1080.
- Sukul, P., Spiteller, M., 2001. Influence of biotic and abiotic factors on dissipating metalaxyl in soil. Chemosphere 45, 941–947.
- Sukul, P., Moza, P.N., Hustert, K., Kettrup, A., 1992. Photochemistry of metalaxyl. J. Agric, Food Chem. 40 (12), 2488-2492.
- Susarla, S., Medina, V.F., McCutcheon, S.C., 2002. Phytoremediation: an ecological solution to organic chemical contamination. Ecol. Eng. 18, 647–658.
- Tanner, C.C., Sukias, J.P.S., 2011. Multiyear nutrient removal performance of three constructed wetlands intercepting tile drain flows from grazed pastures. J. Environ. Qual. 40, 620–633.
- Toxicology Data Network Last Revision Date: 12 September 2003. Hazardous Substances Databank Number: 7061. U.S. National Library of Medicine, 8600 Rockville Pike, Bethesda, MD, United States. Website: http://toxnet.nlm.nih.gov/ (accessed 20.02.11).
- Toxicology Data Network Last Revision Date: 06 August 2002. Hazardous Substances Databank Number: 1003. U.S. National Library of Medicine, 8600 Rockville Pike, Bethesda, MD, United States. Website: http://toxnet.nlm.nih.gov/ (accessed 20.02.11).
- Trapp, S., MacFarlane, J.C., 1995. Plant Contamination: Modeling and Simulation of Organic Chemical Processes. Lewis, Boca Raton, FL.
- U.S. Department of Agriculture, Soil Conservation Service, 1990. SCS/ARS/CES Pesticide Properties Database: Version 2.0 (Summary). USDA-Soil Conservation Service, Syracuse, NY.
- U.S. Department of Interior, Fish and Wildlife Service, 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Resource Publication No. 137. U.S. Government Printing Office, Washington, DC, p. 79.
- U.S. Environmental Protection Agency, 2010. Water quality standards for the state of Florida's lakes and flowing waters; proposed rule. Fed. Regis. 75 (16), 4174–4226, Website: http://www.dep.state.fl.us/water/ wqssp/nutrients/docs/federal/fed_reg_epa_nutrient_criteria_fla.pdf (accessed: 02.01.12).
- U.S. Environmental Protection Agency, 2009. National Primary Drinking Water Regulations. EPA 816-F-09-004.
- U.S. Environmental Protection Agency, 2007. ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. Website: http://www.epa.gov/ecotox/ (accessed 26.02.11).
- Wang, L., Duggin, J.A., 2008. A simulation experiment on the effectiveness of tree and pasture filter strips to remove NO₃–N in lateral soil water flow. J. Environ. Qual. 37, 2145–2154.
- Warren, S.L., Bilderback, T.E., Tyler, H.H., 1995. Efficacy of three nitrogen and phosphorous sources in container-grown azalea production. J. Environ. Hort. 13, 147–151.
- Wheeler, W.B., Stratton, G.D., Twilley, R.R., Ou, L.T., Carlson, D.A., Davidson, J.M., 1979. Trifluralin degradation and binding in soil. J. Agric. Food Chem. 27 (4), 702–706.
- Wilson, P.C., Whitwell, T., Klaine, S.J., 2000. Metalaxyl and simazine toxicity to and uptake by *Typha latifolia*. Arch. Environ. Contam. Toxicol. 39, 282–288.
- Wilson, C.P., Whitwell, T., Klaine, S.J., 2001. Metalaxyl toxicity, uptake, and distribution in several ornamental plant species. J. Environ. Qual. 30, 411–417.
- Wilson, P.C., Whitwell, T., Riley, M.B., 1996. Detection and dissipation of isoxaben and trifluralin in containerized plant nursery runoff water. Weed Sci. 44, 683–688.
- Wilson, P.C., Whitwell, T., Riley, M.B., 1995. Effects of ground cover and formulation on herbicides in runoff water from miniature nursery sites. Weed Sci. 43, 671–677.
- Yao, J.R., Liu, S.Y., Freyer, A.J., Minard, R.D., Bollag, J.M., 1989. Photodecomposition of metalaxyl in an aqueous solution. J. Agric. Food Chem. 37 (6), 1518– 1523.
- Yeager, T., Cashion, G., 1993. Controlled-release fertilizers affect nitrate nitrogen runoff from container plants. HortTechnology 3 (2), 174–177.