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Research Article

Variation in glyphosate effects and accumulation in emergent macrophytes

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Abstract

Invasive aquatic plants can disrupt native biodiversity with considerable ecological impacts. Glyphosate-based herbicides provide one effective option to manage invasive macrophytes, but the variation in glyphosate sensitivity and accumulation in both target and non-target macrophytes is unclear. We performed an outdoor microcosm concentration-response study in which we applied seven glyphosate concentrations (0.1–8% of the Roundup WeatherMAX® formulation, corresponding to 0.5–43.2 g L^{-1} glyphosate) in order to compare sensitivities and accumulation amounts of glyphosate and aminomethylphosphonic acid (AMPA; one degradation product) within and among two invasive emergent plants in North America (*Phragmites australis* and *Typha* × *glauca*), plus a native co-occurring plant (*Typha latifolia*) over a period of 27 days. *Phragmites australis* (dose lethal to 50% of the plant population, LC50 = 0.34 ± 0.03%) exhibited four to five times higher glyphosate sensitivity than *T. latifolia* (LC50 = 1.37 ± 0.13%) and *T.* × *glauca* (LC50 = 1.70 ± 0.17%). Invasive *T.* × *glauca* and native *T. latifolia* exhibited a similar glyphosate response, although individual variation was high within both taxa. 27 days after treatment with 5% glyphosate, a concentration that mimics many real-world applications, *P. australis* retained more glyphosate (348 ± 27 mg kg^{-1} dw) than either *T. latifolia* (102 ± 20 mg kg^{-1} dw; $P < 0.001$) or *T.* × *glauca* (92 ± 12 mg kg^{-1} dw; $P < 0.0001$). Our results suggest that glyphosate response varies among and within emergent aquatic macrophyte taxa, independent of taxon invasiveness. When the goal is to minimize glyphosate exposure of the environment, managers could consider variation in glyphosate response at both individual and taxonomic levels. Moreover, managers should be aware that glyphosate accumulates in macrophytes.

Key words: AMPA, bioaccumulation, herbicide, microcosm, *Phragmites*, toxicity, *Typha*

Introduction

Marshes, ditches, and other semi-aquatic areas are valuable because they provide numerous core ecosystem services including water and food supply, nutrient cycling, and recreation (van der Valk 2006; Maltby and Acreman 2011). While native macrophytes are essential components of healthy aquatic and semi-aquatic ecosystems (Chambers et al. 2008),

invasive macrophytes can threaten ecosystem functions by displacing native species, altering habitats, and disrupting food webs (Pimentel et al. 2005; Rejmankova 2011; Pyšek et al. 2012). Control of invasive plants with the goal of restoring native biodiversity is therefore a primary activity of wetland managers (Kettenring and Adams 2011).

Land managers have several options for controlling invasive macrophytes. These include mechanical and physical removal (e.g. cutting, mowing, rolling), biological treatments (e.g. herbivores), and chemical control with herbicides (Ontario Ministry of the Environment 2009; Wagner et al. 2017). Herbicides, particularly glyphosate, are globally the most common management tool for invasive plants (Kettenring and Adams 2011). Glyphosate is non-selective and is rapidly translocated from treated leaves into stems, roots and rhizomes (Solomon and Thompson 2003), and is therefore an effective control agent for many perennial plants with large below-ground biomass (Crowe et al. 2011; Linz and Homan 2011).

In North America, two commonly managed invasive plants are the emergent, rhizomatous perennials *Phragmites australis* (Cav.) Trin. ex Steud. and *Typha* × *glauca* Godr. (Linz and Homan 2011; Hazelton et al. 2014). Over the past two centuries the Eurasian *P. australis* lineage has invaded aquatic and semi-aquatic ecosystems across North America (Galatowitsch et al. 1999; Martin and Blossey 2013). *Typha* × *glauca*, a hybrid of native *Typha latifolia* L. and introduced *Typha angustifolia* L. (Ciotir et al. 2013; Ciotir and Freeland 2016), is a problematic invader in midwestern and eastern North America (Galatowitsch et al. 1999; Tuchman et al. 2009). *Phragmites australis* and *T.* × *glauca* share key invasive traits such as forming tall, dense monotypic stands, spreading rapidly through clonal growth, and leaving behind abundant litter following senescence, all of which can alter habitat structure and function (Zedler and Kercher 2004).

When glyphosate is used to control *P. australis* or *T.* × *glauca*, managers select a concentration based on recommendations from the formulated product label (e.g. Roundup WeatherMAX®; Monsanto Canada Inc. 2017). The label includes a wide range of glyphosate concentrations for broadcast spray applications on *Phragmites* (2–8 L ha^{-1} in 100–500 L ha^{-1} water) and *Typha* (grouped under "Other perennials"; 4.67–8 L ha^{-1} in 100–300 L ha^{-1} water), and a single, generic rate (1.34%; see Table 1) for all harder-to-control perennials using hand-held spray equipment (Monsanto Canada Inc. 2017), which may not provide sufficient information for managers to select a particular effective concentration for their target taxon.

Selecting the most appropriate dose to use in an ecosystem with both target taxa and protected non-target taxa requires information about their relative sensitivities. Earlier studies have focused on determining glyphosate efficacy for either *Phragmites* or *Typha* at one or a few concentrations, using set-ups of different scales (experimental or field trial) and variable monitoring periods (two weeks to two years). A single application of 1.5–4.7%

Table 1. Rationale for applied nominal Roundup WeatherMAX® treatment concentrations in the microcosm concentration-response experiment.

Treatment [%]	Glyphosate concentration [mg L^{-1}]	Rationale
8.00	43.2	Maximum rate allowed by the label for *Phragmites*, and *Typha* (grouped under "Other Perennials"), calculated from the advised maximum rate of 8 L ha^{-1} Roundup Weathermax® in 100 L ha^{-1} water when using aerial application equipment (Monsanto Canada Inc. 2017). Highest expected realistic exposure.
5.00	27.0	Rate used to control *Phragmites* in some Ontario ecosystems (Ontario Ministry of Natural Resources 2011; Howell 2017; Tozer and Mackenzie 2019). Direct exposure most representative of real-world applications.
1.56	8.4	Minimum rate advised by the label for *Typha* (grouped under "Other Perennials"), calculated from the advised minimum rate of 4.67 L ha^{-1} Roundup Weathermax® in 300 L ha^{-1} water when using aerial application equipment (Monsanto Canada Inc. 2017).
1.34	7.2	Advised label rate for handheld spray equipment for all harder-to-control perennials (Monsanto Canada Inc. 2017).
0.40	2.1	Minimum rate advised by the label for *Phragmites*, calculated from the advised minimum rate of 2 L ha^{-1} Roundup Weathermax® in 500 L ha^{-1} water when using aerial application equipment (Monsanto Canada Inc. 2017).
0.23	1.2	Lower concentration that may imitate spray drift on non-target plants adjacent to target spray area.
0.10	0.5	Lower concentration that may imitate spray drift on non-target plants adjacent to target spray area.
0	0	Control treatment: Glyphosate-free municipal tap water.

glyphosate controlled 63–96% of *Phragmites* plants (Fell et al. 2006; Derr 2008a, b; Cheshier et al. 2012; Rapp et al. 2012), whereas glyphosate concentrations of 1.25–15% controlled 58–96% of *Typha* plants (Comes and Kelley 1989; Linz et al. 1992; Kay 1999). These studies have contributed to our knowledge on *Phragmites* or *Typha* control, but it remains difficult to draw conclusions about the relative sensitivities of *Phragmites* and *Typha* from comparisons of studies that used different set-ups and monitoring periods. Moreover, it is unclear how much glyphosate may be retained in sprayed plant tissues, and if retained amounts vary among taxa. Glyphosate bound in plant material degrades more slowly than in soil (Mamy et al. 2016), and may thus persist longer in the soil environment, with potential effects on growth and disease resistance of recolonizing plants that come into contact with soil residues (Neumann et al. 2006; Tesfamariam et al. 2009; Van Bruggen et al. 2018). These knowledge gaps may impact our ability to balance effective control of target plants against minimal impact on non-target plants.

Sensitivity to herbicides varies among plant taxa due to differences in interception, absorption, translocation from treated leaves to roots and rhizomes, and metabolism (Sandberg et al. 1980; Cedergreen et al. 2004). *Phragmites* is a grass with broad leaves and rigid, hollow stems (Mal and Narine 2004), whereas *Typha* produces basal, linear leaves (Grace and Harrison 1986), and morphological differences such as these may lead to differences in glyphosate interception and accumulation. Moreover, *Typha* has an earlier growing season than *Phragmites* (Mason and Bryant 1975), and control efficacy can be influenced by growth stage (Knezevic et al. 2013). Response to glyphosate may also vary among congeneric taxa. There

is evidence of heterosis in *T.* × *glauca* (Bunbury-Blanchette et al. 2015; Zapfe and Freeland 2015), and this could further manifest as decreased herbicide sensitivity relative to its parent species *T. latifolia*. Furthermore, *T.* × *glauca* absorbs less glyphosate through rhizomes than *T. latifolia* (Zheng et al. 2017), which could result in decreased sensitivity.

In this study we assess inter- and intra-generic differences in glyphosate sensitivity and accumulation in emergent macrophytes using the model taxa *Phragmites australis*, *Typha* × *glauca* and *Typha latifolia*. We hypothesize that 1) *Phragmites* and *Typha* exhibit inter-generic variation in glyphosate sensitivity and accumulation, and 2) *Typha* exhibits intra-generic variation in glyphosate sensitivity and accumulation. In particular, we predict that 1) *Phragmites* exhibits higher glyphosate sensitivity and accumulation than *Typha* due to its hollow above-ground shoots and non-fleshy leaves, and 2) *T. latifolia* exhibits higher glyphosate sensitivity and accumulation than *T.* × *glauca* based on its ability to absorb more glyphosate in rhizomes. Understanding variability in emergent macrophytes' responses to glyphosate can improve the accuracy of application rate calculations and risk assessments as part of a balanced management approach that effectively reduces invasive species while minimizing risks to non-target plants.

Materials and methods

Macrophyte collection and propagation

We collected *T. latifolia* and *T.* × *glauca* seeds from natural populations in Ontario and Nova Scotia, Canada (Supplementary material Table S1) in fall 2017 and stored them at 4 °C (Tisshaw 2019). Provisional taxonomic identifications based on morphology were confirmed with microsatellite genotyping following methods of Kirk et al. (2011). In February 2018, we prepared *Typha* seeds following the methods of Ahee et al. (2015), and placed them in sterile Petri dishes half-filled with de-ionized water in a greenhouse to germinate. Germinated seedlings were grown and fertilized following the methods of Tisshaw (2019). Germinated seedlings from each Petri dish were transferred into 200-cell plug trays filled with soil (Sunshine professional growing mix #15, Sungro, Agawam, U.S.A.), which were placed in flats filled approximately half-full with water, and covered with clear plastic domes for the first three weeks to maintain humidity. After five weeks, we started weekly additions of 100 mL of 2% water-soluble 20-20-20 N-P-K general purpose fertilizer (Peters Professional®, Scotts, Marysville, U.S.A.) to flats. In mid-May 2018, we transplanted plants into 7.6 L plastic pots filled with soil (Sunshine professional growing mix #15, Sungro, Agawam, U.S.A.).

Due to germination difficulties, *P. australis* was collected as shoots in late May 2018 (Table S1). *Phragmites australis* was provisionally identified in the field based on morphological characteristics (Swearingen et al. 2012)

and subsequently genotyped to confirm their identification as the invasive taxon following methods of Paul et al. (2010). Partially submerged shoots were clipped above soil level but below the water surface, so that the clipped shoot had at least two nodes with roots. *Phragmites* shoots appeared stressed following their transport, so we planted two shoots in each of the 56 7.6 L plastic pots as insurance against mortality. Pots were filled with soil (Sunshine professional growing mix #15, Sungro, Agawam, U.S.A.), so that shoots had at least two nodes with roots below the soil surface.

Experimental set-up

We conducted the experiment in an outdoor research facility at Trent University, Peterborough, Ontario, Canada, using microcosms (artificial multi-species test systems that simulate characteristics of the natural environment for the purposes of ecotoxicological effects assessment; Nordberg et al. 2009). We used a randomized complete block design with seven blocks of eight microcosms each, arranged in a 7 × 8 matrix, which gave us seven replicates of each of our seven glyphosate treatments, and seven replicates of the glyphosate-free control (Table 1, Figure S1). Each block contained one replicate of each glyphosate treatment and the control treatment, and treatments were randomized within each block. Each microcosm was a 67 L plastic bucket, filled with 19 L glyphosate-free municipal tap water, and holding three 7.6 L plastic pots, which collectively contained one each of *T. latifolia*, *T.* × *glauca* or *P. australis*. The *Phragmites* pots were transferred to the microcosms 54–55 days prior to dosing to acclimatize; *Typha* pots were added 63–64 days prior to dosing. The slight variation in acclimation periods reflects the times at which material from different taxa was available. The water level in each microcosm was maintained at approximately 3 cm above the interior pots to keep the soil submerged. In addition, we grew untreated control treatments in pots located approximately two meters from the experimental setup, to confirm that our plants contained no background glyphosate contamination (field control pots). These field controls included ten *Phragmites* pots, eight *T.* × *glauca* pots and seven *T. latifolia* pots (numbers vary due to limited availability). Experimental *Phragmites* and *Typha* plants were mature and actively growing, but had not formed any flowers or fruits.

Glyphosate treatments

On July 19, 2018, we applied glyphosate to the microcosms (Roundup WeatherMAX® with Transorb 2 Technology Liquid Herbicide formulation; 540 g active ingredient L^{-1}; Monsanto Canada Inc., 900 One Research Road, Winnipeg, MB, Canada, R3T 6E3). Roundup WeatherMAX® contains 48.8% active ingredient glyphosate as potassium salt (potassium N-[(hydroxyphosphinato)methyl]glycine). The remaining 51.2% includes

surfactant, water and other minor ingredients (Monsanto Canada Inc. 2018), the specifics of which were not disclosed by the manufacturer upon request (Monsanto Canada *pers. comm.* on 10 June 2019). Roundup WeatherMAX® is registered for *Phragmites* and *Typha* control in locations such as roadside ditches. It is not labelled for use where surface water is present at the time of application (Monsanto Canada Inc. 2017), but due to the biology of *Phragmites* and *Typha* any location where these plants are thriving will likely have some standing water at some point during the year. Specific formulations of glyphosate-based herbicides vary (for example, Roundup Custom® is formulated for use over surface waters, and includes a different surfactant than Roundup WeatherMAX®). However, this study focuses on the active ingredient glyphosate and one primary degradation product, aminomethylphosphonic acid (AMPA), and we consider those results to probably be generalizable among glyphosate-based herbicides.

We applied concentrations of 8.00, 5.00, 1.56, 1.34, 0.40, 0.23 and 0.1% solutions, and a 0% control treatment (see Table 1 for rationale and Table S2 for respective measured concentrations). Methylated seed oil was added at 1% v v^{-1} to each prepared glyphosate solution to increase wetting and thereby facilitate penetration into plant tissues (Monsanto Canada Inc. 2017), which is common practice for field applications in Ontario (Howell 2017; Ontario Phragmites Working Group 2015; Tozer and Mackenzie 2019). Treatment concentrations are hereafter expressed in spray rates [%], as these are most relevant to the hand-held and backpack spray operations that we address in this study (Howell 2017; Monsanto Canada Inc. 2017).

We sprayed glyphosate on the plant foliage to imitate treatment procedures applied in the field (Linz and Homan 2011; Quirion et al. 2018). Each microcosm was dosed with 50 mL of one of the eight treatments by spraying evenly over each plant canopy using motorized hand-gun equipment (Instapark® AHS-803 Rechargeable Electronic Water Mist Sprayer). Initial trials determined a volume of 50 mL as appropriate amount to fully wet the plants but not to the point of run-off ("spray-to-wet" procedure as advised by the label; Monsanto Canada Inc. 2017). Based on 50 mL volume of spray solution and area of each microcosm (2.34×10^{-6} ha), our applied glyphosate treatments of 0.1–8% translated into applications rates of 1.15–92.22 kg glyphosate ha^{-1}. We minimized potential spray drift during application by surrounding each microcosm with an enclosure made from four wooden posts, wrapped with a single-use plastic sheet. We discarded each sheet after each treatment, moved the posts to the next replicate, and used a new sheet to create the enclosure for the next microcosm, minimizing contact between each enclosure and the surrounded foliage to allow spray to reach all parts of the treated plants. On the application day, maximum air temperature was 27.1 °C, without rain or wind (Table S3), sunny with scattered, passing clouds from 13:00–15:00. The experiment ran for 27 days until 15 August 2018, because first symptoms of glyphosate

exposure may be visible only after 7–10 days (Baylis 2000; Monsanto Canada Inc. 2017).

Experimental conditions

Throughout the 27-day experimental period, air temperature was on average 22.1 °C (min. 10.1 °C, max. 33.4 °C), with 14 rain days with an average of 3.1 mm precipitation (min. 0.2 mm, max. 13.1 mm) (see Table S3 for details). Dissolved oxygen in the microcosm water was on average 3.0 mg L^{-1} (min. 0.3 mg L^{-1}, max. 9.5 mg L^{-1}), pH on average 7.0 (min. 6.4, max. 7.9), salinity on average 0.2 psu (min. 0.1 psu, max. 0.4 psu), and temperature on average 24.7 °C (min. 19.5 °C, max. 29.7 °C) (see Figure S2 for details).

Experimental endpoints

At 27 days after spraying we quantified the total number of shoots for each plant by counting all above-ground shoots (alive and dead), and quantified the number of dead shoots by counting all above-ground shoots that were completely brown (no green areas on any leaf or stem parts). We calculated the proportion of dead shoots as number of dead shoots divided by total number of shoots, as a proxy for the proportion of dead tissue. Proportion of dead shoots was a more reliable endpoint than biomass assessments, so we used this endpoint for subsequent analyses (see Supplementary material Appendix 1 for details).

At 27 days post-exposure, all plant tissue was harvested from four different plants from each taxa (except n = 3 for TL at 5%; one replicate was omitted from analyses as genotyping indicated incorrect morphological taxon identification) that had been exposed to each of 8% and 5% glyphosate (representing the maximum label rates and a commonly used rate; Table 1). Samples were immediately frozen at −20 °C, and sent on ice to the Agriculture and Food Laboratory, University of Guelph (AFL). In addition, for each taxon approximately 200 g of plant tissue from a pooled sample of leaf fragments from the field control pots was sent for analysis. At AFL, glyphosate and AMPA (one common degradation product) residues were analysed using Liquid chromatography-mass spectrometry/Mass spectrometry (LC-MS/MS). For this, the laboratory prepared an aqueous extract of a homogenized subsample of plant material, which was then acidified and separated from co-extractives using solid phase extraction. The LC-MS/MS system employed a cation guard column (Micro-Guard Cation-H cartridge 30 × 4.6 mm) for chromatographic separation, a mobile phase A (0.1% formic acid in nanopure grade H_2O) and B (acetonitrile), with a flow rate of 1 mL min^{-1}, a total run time of 12 min, and retention times of 0.9 min for glyphosate and 4.2 min for AMPA. Auto-sampler temperature was set at 8 °C, and the injection volume was 50 µL. Column oven temperature was set at 20 ± 3 °C.

While our main focus was on accumulation in above-ground tissues, we wanted to also estimate amounts of glyphosate and AMPA in water and sediment, and therefore we collected 700 mL of pooled microcosm water from each treatment (100 mL from each of seven replicates) at one hour and 27 days post-spray, and 42 g fresh weight of pooled sediment for each treatment (6 g from each of seven replicates) 27 days post-spray. Water and sediment samples were immediately frozen at −20 °C, and sent on ice to AFL to be analysed for glyphosate and AMPA residues following the same procedure as described above.

Laboratory detection limits for glyphosate and AMPA are 0.005 mg kg^{-1} fresh weight in plant tissue and soil, and 0.001 mg L^{-1} in water; quantification limits are 0.02 mg kg^{-1} fresh weight in plant tissue and soil, and 0.008 mg L^{-1} in water. Reported analytical quality control recoveries were on average (± standard error) 97.7 ± 2.8% for glyphosate and 111.5 ± 4.3% for AMPA.

Plant tissue and soil samples were also analysed for % dry matter at AFL. For this, AFL dried plant and soil subsamples to constant weight for 24–48 hours at 105 °C in a drying oven (Fisher Science Isotemp Oven, Model 615F), and calculated the ratio of dry weight and fresh weight of the same sample, which was reported as % dry matter for each sample.

Statistical analyses

Statistical analyses were conducted with R (R Core Team 2016). We used the package "drc"(Ritz and Streibig 2016) to model concentration-response curves to the binomial data and estimate the median lethal concentration (LC50; concentration estimated to be lethal to 50% of test plants) for each macrophyte taxon based on the curves and their associated confidence bounds. For each macrophyte, we tested a selection of concentration-response models using the "mselect" function, which compares models using the following criteria: log likelihood value, Akaike's information criterion (AIC), estimated residual standard error, and lack-of-fit test p-value (Ritz et al. 2015). We chose the "best" model for relative LC50 and confidence interval estimation, and evaluated it using statistical and visual diagnostics tools for model fit, normality of residuals, and variance homogeneity (Stephenson et al. 2000; Environment and Climate Change Canada 2007). All model assumptions were met on untransformed data. The two-parameter log-logistic function ("LL.2") was best for *P. australis* and *T. latifolia*, while the two-parameter Weibull function ("W1.2") was best for *T.* × *glauca*. Similarly good models (difference of AIC relative to $AIC_{min} < 2$; Burnham and Anderson 2010) were evaluated for comparison and resulted in similar LC50 estimates (Table S4).

Glyphosate and AMPA accumulation were determined in fresh tissue, and data was converted to concentrations in dry tissue using the quantified % dry matter for each sample. We analysed glyphosate and AMPA

concentrations in plant tissues with two-way analysis of variance (ANOVA; "aov" function in package "stats"), with "Macrophyte" and "Treatment concentration" as independent factor variables. ANOVA models were evaluated using statistical and visual diagnostics tools for model fit, normality of residuals, and variance homogeneity (Environment and Climate Change Canada 2007). We evaluated additive models against interactive models in cases of similar good fit (difference of AIC relative to $AIC_{min} < 2$; Burnham and Anderson 2010). Additive models were more strongly supported due to fewer parameters and insignificant interaction ($P > 0.05$). Tukey Honest Significant Differences ("TukeyHSD" function in package "stats") was used to perform multiple pairwise-comparisons between the means of groups. Moreover, we used linear regression analysis ("lm" function in package "stats") to assess if there is a relationship between applied glyphosate concentration and accumulated glyphosate and AMPA in plant tissues for each macrophyte taxa. We considered results different at $P < 0.05$ (Dushoff et al. 2019).

Results

Glyphosate effects

All three plant taxa showed visible effects of glyphosate exposure including foliar speckling, chlorosis, and necrosis, as early as four days post-exposure (Figure 1). Glyphosate sensitivity varied among taxa (Table 2). Glyphosate sensitivity of *P. australis* (LC50 = 0.34 ± 0.03%) was four times higher compared to *T. latifolia* (LC50 = 1.37 ± 0.13%), and five times higher than *T.* × *glauca* (LC50 = 1.70 ± 0.17%) at 27 days post-exposure (Figure 2). *Typha* × *glauca* and *T. latifolia* were not different in their sensitivity to glyphosate based on their estimated LC50s whose confidence limits overlap (Figure 2).

Glyphosate and AMPA accumulation

After 27 days, above-ground tissues of all three taxa contained detectable levels of glyphosate and AMPA (Figure 3). *Phragmites australis* accumulated on average (± standard error) 348 ± 27 and 609 ± 94 mg glyphosate kg^{-1} dry tissue, and 9 ± 1 and 13 ± 2 mg AMPA kg^{-1} dry tissue treated at 5% and 8%, respectively. *Typha* × *glauca* accumulated on average 92 ± 12 and 83 ± 10 mg glyphosate kg^{-1} dry tissue, and 2 ± 1 and 2 ± 0 mg AMPA kg^{-1} dry tissue treated at 5% and 8%, respectively. *Typha latifolia* accumulated on average 102 ± 20 and 204 ± 88 mg glyphosate kg^{-1} dry tissue, and 2 ± 0 and 3 ± 1 mg AMPA kg^{-1} dry tissue treated at 5% and 8%, respectively.

Glyphosate accumulation in plant tissues varied among macrophyte taxa (ANOVA, $F_{2,19} = 22.7$, $P < 0.0001$) and between the two glyphosate treatments applied (5% and 8%; $F_{1,19} = 5.3$, $P < 0.05$). A Tukey post hoc test determined that *P. australis* accumulated more glyphosate than *T.* × *glauca*

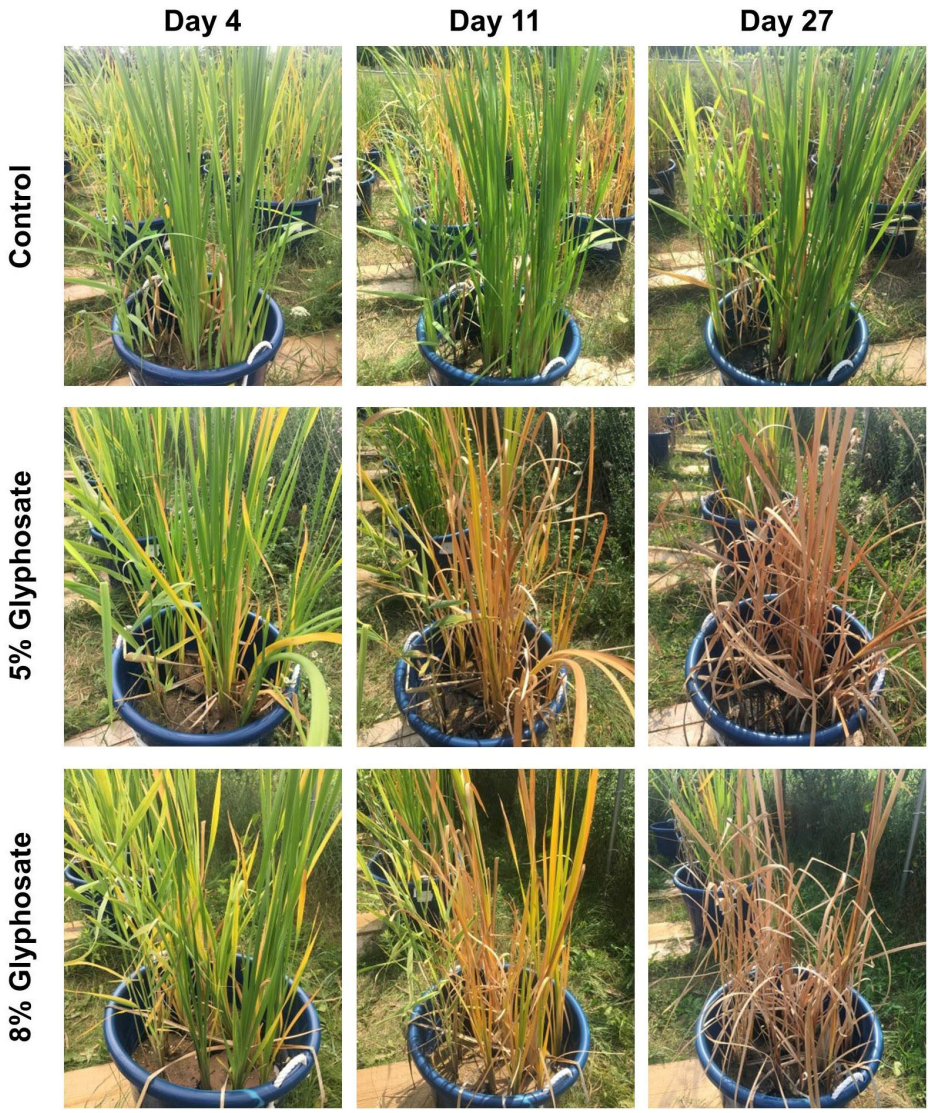

Figure 1. Representative replicates of the control treatment (tap water) and glyphosate (Roundup WeatherMAX® formulation) concentrations of 8% (43.2 g L^{-1}) and 5% (27.0 g L^{-1}) on day 4, 11 and 27 post-exposure. Each replicate (blue bucket) contained one pot of *Typha latifolia* (in the back), one pot of *Typha* × *glauca* (in the front), and one pot of *Phragmites australis* (on the left), and was sprayed with 50 mL of the respective treatment solution evenly covering above-ground plant tissues. Photos by Verena Sesin.

Table 2. Average proportion of dead shoots ± standard error (SE) for the three macrophytes *Phragmites australis*, *Typha latifolia* and *Typha* × *glauca* at 27 days post-exposure to 0–8% glyphosate (Roundup WeatherMAX® formulation)

Treatment [%]	Average proportion of dead shoots ± SE		
	Phragmites australis	*Typha latifolia*	*Typha* × *glauca*
8.00	1.00 ± 0.00	0.96 ± 0.03	0.81 ± 0.09
5.00	0.97 ± 0.02	0.80 ± 0.08	0.87 ± 0.04
1.56	0.96 ± 0.03	0.58 ± 0.11	0.46 ± 0.11
1.34	0.88 ± 0.09	0.70 ± 0.04	0.42 ± 0.10
0.40	0.64 ± 0.06	0.13 ± 0.06	0.03 ± 0.03
0.23	0.33 ± 0.09	0.02 ± 0.02	0.04 ± 0.12
0.10	0.18 ± 0.04	0.02 ± 0.02	0.00 ± 0.00
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

($P < 0.0001$) and *T. latifolia* ($P < 0.001$), whereas both *Typha* taxa accumulated similar amounts ($P > 0.05$).

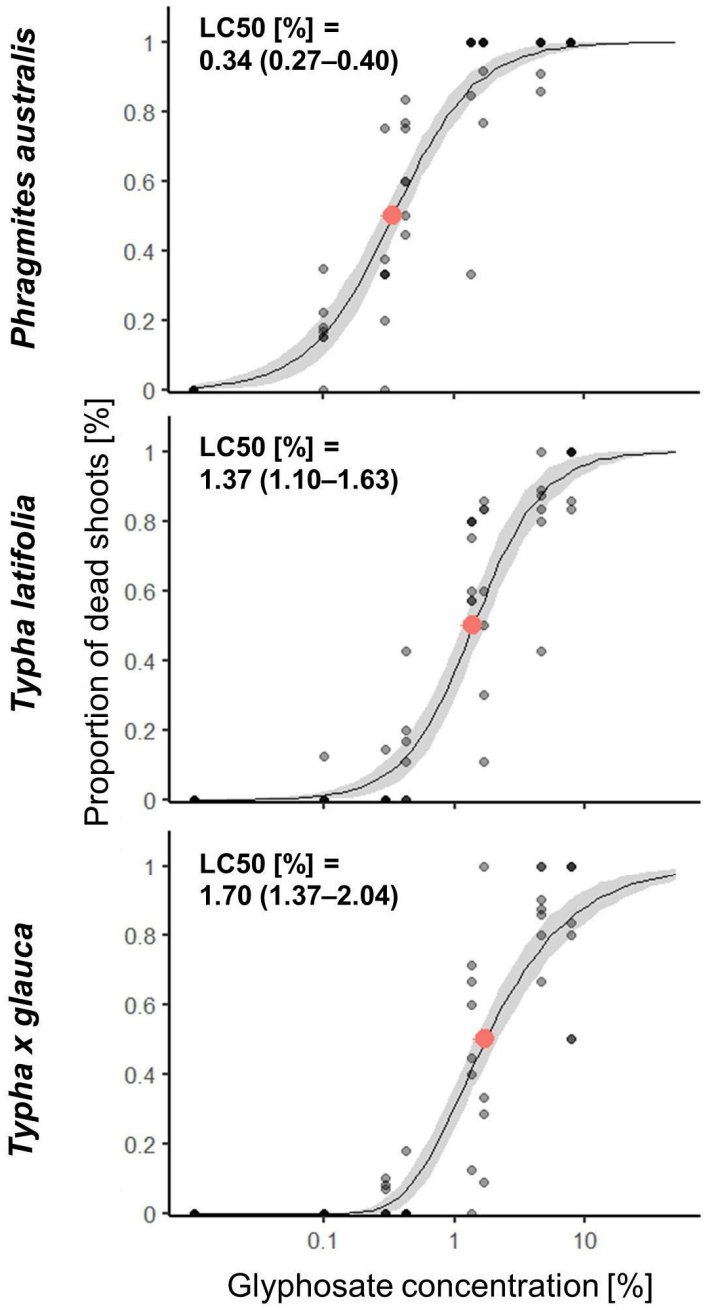

Figure 2. Concentration-response relationships for *Phragmites australis* (top graph), *Typha latifolia* (middle graph) and *Typha* × *glauca* (bottom graph) based on the proportion of dead shoots observed 27 days post-exposure to 0–8% glyphosate (Roundup WeatherMAX® formulation). Each treatment was replicated seven times per taxa, except *T. latifolia* at 5% (n = 6). Measured concentrations were used for modelling (Table S2). Grey points represent the replicates and are darker when points overlap; grey shading illustrates the 95% confidence bounds of the concentration-response model; solid red dots represent LC50 estimates; LC50 estimates on top left corner of each graph are given with lower and upper 95% confidence limits.

Likewise, AMPA accumulation in plant tissues varied among macrophyte taxa (ANOVA, $F_{2,19} = 44.7$, $P < 0.0001$) but not between the two glyphosate treatments applied (5% and 8%; $F_{1,19} = 2.1$, $P > 0.05$). A Tukey post hoc test determined that *P. australis* accumulated more AMPA than *T.* × *glauca* ($P < 0.0001$) and *T. latifolia* ($P < 0.0001$), whereas both *Typha* taxa accumulated similar amounts ($P > 0.05$).

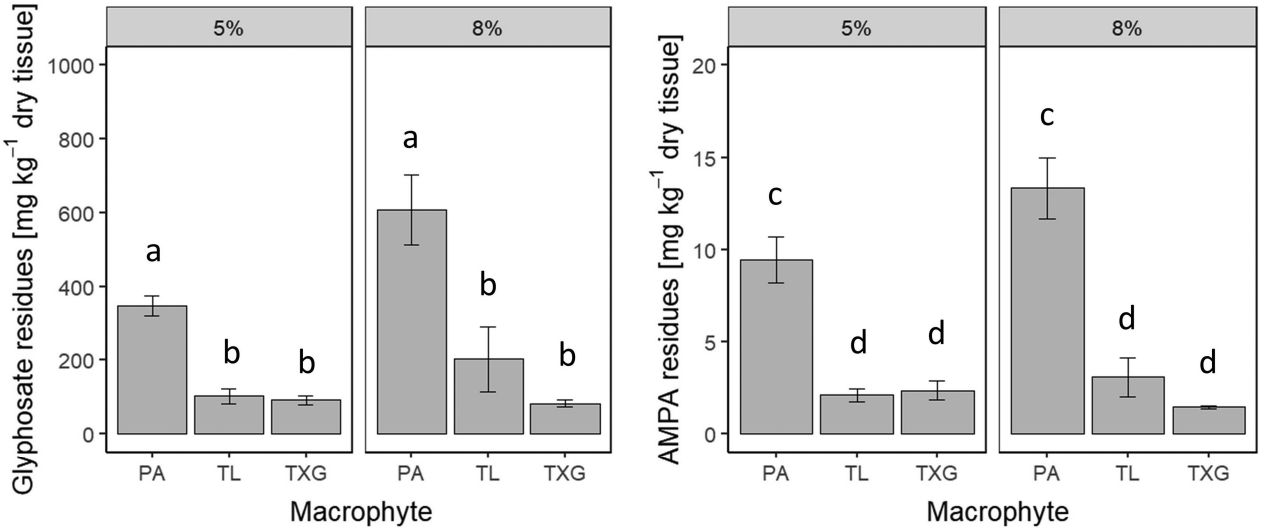

Figure 3. Average residues of glyphosate (left) and aminomethylphosphonic acid (AMPA; right) detected in above-ground plant tissues of the three macrophytes *Phragmites australis* (PA), *Typha latifolia* (TL) and *Typha* × *glauca* (TXG) 27 days post-exposure to 8% and 5% glyphosate solutions (Roundup WeatherMAX® formulation). Four replicates per taxon and treatment, except for TL at 5% (n = 3); error bars represent standard errors of the means; letters a-b (left plot) and c-d (right plot) indicate significant differences in residues among taxa. Note y-axes have different scales.

A linear regression analysis for each taxon detected a weak relationship between applied glyphosate concentration and accumulated glyphosate for *P. australis* ($F_{1,6} = 7.0$, adj. $R^2 = 0.46$, $P < 0.05$), but not for *T.* × *glauca* ($F_{1,6} = 0.3$, adj. $R^2 = 0.00$, $P > 0.05$) or *T. latifolia* ($F_{1,5} = 0.9$, adj. $R^2 = 0.00$, $P > 0.05$). There was no relationship between applied glyphosate concentration and accumulated AMPA for *P. australis* ($F_{1,6} = 3.5$, adj. $R^2 = 0.26$, $P > 0.05$), *T.* × *glauca* ($F_{1,6} = 2.9$, adj. $R^2 = 0.22$, $P > 0.05$) and *T. latifolia* ($F_{1,5} = 0.6$, adj. $R^2 = 0.00$, $P > 0.05$).

Glyphosate residues in *Typha* collected from the field control were below the quantification limit (< 0.02 mg kg^{-1} fresh tissue). Field control *Phragmites* contained low glyphosate levels (0.063 mg kg^{-1} fresh tissue). Detected glyphosate and AMPA residues in microcosm water 1 h and 27 days post-exposure increased with treatment concentration and decreased with time (Figure S3). In the 8% treatment we detected a maximum of 14 mg L^{-1} glyphosate in microcosm water at 1 h post-exposure, and 9 mg L^{-1} at 27 days post-exposure (Figure S3). Detected glyphosate and AMPA residues in microcosm sediment 27 days post-exposure increased with treatment concentration, with a detected glyphosate maximum of 8 mg kg^{-1} dw in the 8% treatment (Figure S4).

Discussion and conclusions

Our study assessed the response of emergent macrophytes to a range of glyphosate concentrations and measured differences in glyphosate sensitivity and accumulation capacity among taxa. *Phragmites australis* exhibited four to five times greater sensitivity to glyphosate than *T. latifolia* and *T.* × *glauca* based on LC50 estimates, and accumulated more glyphosate and AMPA in above-ground tissues. Invasive *T.* × *glauca* and native

T. latifolia had similar glyphosate sensitivity and accumulation, although sensitivity of individuals in both taxa was variable. Our study is the first to quantify inter- and intra-generic variation in response to glyphosate in emergent macrophytes and can inform glyphosate-based management of invasive macrophytes.

Our results support the hypothesis that *Phragmites* and *Typha* exhibit inter-generic variation in glyphosate sensitivity and accumulation. This variation may reflect differing above-ground morphology between *Phragmites* and *Typha*, such as leaf size, arrangement and numbers, which could cause variation in glyphosate interception and retention (Gottrup et al. 1976; Baril et al. 2005). Glyphosate absorption could be higher in *Phragmites* than *Typha* leaves based on *Phragmites*' thinner epicuticular wax and cuticle (Al-Hadeethi et al. 2016) which could lead to increased uptake. Moreover, plants can vary in their abilities to translocate glyphosate (Sandberg et al. 1980) and to metabolize it to AMPA and other degradation products such as sarcosine and glycine (Reddy et al. 2008; Duke 2011; Rojano-Delgado et al. 2012), although translocation and degradation rates have not been quantified for *Phragmites* and *Typha*. Increasing glyphosate concentration from 5% to 8% was weakly related to increased accumulation in *P. australis*, but not in either *Typha* taxon, which suggests that some taxa could have a threshold of maximum glyphosate uptake, which appears lower in *Typha* than *Phragmites*. While *Phragmites* exhibited higher glyphosate sensitivity and accumulation than *Typha* as predicted, further study is needed to determine whether this is attributable to above-ground morphology or glyphosate translocation and degradation ability.

A previous study suggested that glyphosate sensitivity could vary between *Typha* taxa (Zheng et al. 2017), but we measured no difference in glyphosate sensitivity or accumulation between *T.* × *glauca* and *T. latifolia*. This discrepancy may reflect our assessment of glyphosate sensitivity from the above-ground responses of live, mature *Typha* plants. Zheng et al. (2017) measured passive absorption of dissolved glyphosate on oven-dried pulverized rhizome and shoot material, and we did not measure below-ground root and rhizome glyphosate sensitivity and accumulation in this study. Macrophytes can also be highly variable in their above-ground response to pesticides (Arts et al. 2008), requiring a large sample size to detect small differences among taxa. The high variation in glyphosate response that we quantified among individuals of each taxon exposed to the same concentration might be at least partially explained by genetic variation among populations (Boutin et al. 2010), because individuals were collected from multiple populations (Table S1). High standard errors within treatments for both the sensitivity and accumulation data could also reflect uneven foliar spray coverage. We aimed to spray all plants evenly within each microcosm, but spray patterns can be variable with hand-operated equipment (Cornish and Burgin 2005). Some plant foliage was

also overlapping, potentially leading to slight variations in the dose received by each plant, but the seven replicate microcosms for each glyphosate treatment should account for this source of variation. Genetic variation among target plants and uneven spray coverage among individual plants both reflect conditions encountered in "real-world" scenarios.

Several earlier studies have investigated control efficacy for either invasive *Phragmites* or *Typha* at up to three glyphosate rates per study (Comes and Kelley 1989; Linz et al. 1992; Kay 1999; Fell et al. 2006; Derr 2008a, b; Cheshier et al. 2012; Rapp et al. 2012). Our research expands this knowledge by comparing sensitivities of both *Phragmites* and *Typha* to a range of seven glyphosate concentrations, and quantifying how much glyphosate can be retained in tissues when sprayed with two "realistic" glyphosate concentrations using application methods that mirror "real-world" spray operations. Our results suggest that a higher concentration of glyphosate is needed for *Typha* control than for *Phragmites* control. The 5% application, commonly used in *Phragmites* management (Table 1), may not treat invasive *Typha* patches as effectively as an 8% treatment. In our study, even the 8% application did not eliminate all *Typha* plants. A different herbicide (e.g. imazapyr) may be more suitable for *Typha* control, although efficacy of any herbicide may vary with environmental parameters, plant growth stage, and application timing (Coetzer et al. 2001; Waltz et al. 2004; Faccini and Puricelli 2007).

Following plant control activities, glyphosate is thought to be removed quickly from the environment through binding to soil and subsequent microbial degradation (Rueppel et al. 1977; Sviridov et al. 2015), but its degradation can be slowed when bound in plant residues (Mamy et al. 2016). If glyphosate were trapped in plant material such as shoots and rhizomes, it may not be readily metabolized (Mamy et al. 2016; Kilbride and Paveglio 2001). Our study found that glyphosate from spray application can be retained in *Phragmites* and *Typha* shoot tissues for at least 27 days post-exposure. It is unclear how long glyphosate can persist in shoot and rhizome plant material, and to what extent these residues are bioavailable. However, there is some evidence that plant-bound residues could affect the growth and disease resistance of recolonizing plants that come into contact with them (Neumann et al. 2006; Tesfamariam et al. 2009). Both *Phragmites* and *Typha* produce large amounts of slowly decomposing biomass (Mason and Bryant 1975; Ágoston-Szabó and Dinka 2008), which if not removed from the treatment area, could potentially leach glyphosate into the surrounding environment (Neumann et al. 2006; Tesfamariam et al. 2009).

If the goal of glyphosate-based management of invasive macrophytes is to effectively control invasives while minimizing harm to non-target plants, then best practices should consider the variation in glyphosate sensitivity and retention among and within plant taxa. Our findings have three clear implications for glyphosate-based invasive plant control. First,

similar glyphosate response of invasive and native *Typha* implies that selective management of invasive *Typha* in a mixed stand with native *T. latifolia* requires a targeted application method, such as wicking or wiping, to limit detrimental effects on the non-target native plant. However, wicking and wiping are labour-intensive methods that may only be feasible for treating small patches, the edges of larger patches, or cases where invasive shoots grow next to endangered native species. Second, minimum effective concentrations of glyphosate for control of invasive plants should be informed by taxon-specific assessments, to account for inter-specific variation in glyphosate sensitivity. Third, managers who are handling or disposing of decaying plant material following glyphosate application should be aware that sprayed plant tissue retains glyphosate, and that the potential for subsequent release of glyphosate from decaying plant material is unclear. Our results are therefore relevant to glyphosate-based management of invasive plants that aims to effectively control target plants while minimizing risks to non-target species. We propose that further research is undertaken to quantify glyphosate retention in below-ground plant structures, and to assess if treated plant tissues could release bioavailable glyphosate into surrounding soil or surface water. We are confident that our research will serve as a base for these future studies on glyphosate fate and persistence in vegetated ecosystems.

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Data Accessibility

The data that support the findings of this study are openly available in figshare at https://doi.org/10.6084/m9.figshare.11900139

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Supplementary material

The following supplementary material is available for this article:

Table S1. Source of macrophytes.
Table S2. Confirmation of glyphosate treatment concentrations.
Table S3. Weather information.
Table S4. Comparison of modelling estimates.
Figure S1. Experimental microcosm set-up.
Figure S2. Microcosm water quality data.
Figure S3. Glyphosate and AMPA residues in microcosm water.
Figure S4. Glyphosate and AMPA residues in microcosm sediment.
Appendix 1. Biomass measurements.
Appendix 2. References.