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Physical and physiological pathways of off-target triclopyr movement and associated non-target injury following basal bark application

Research Article

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Abstract

Basal bark treatment with triclopyr butoxyethyl ester is used to control woody invasive plants, including Brazilian peppertree (*Schinus terebinthifolia* Raddi). However, the ester formulation cannot be applied where standing water is present, which includes wetlands where *S. terebinthifolia* is found. In 2009, a low-volatile acid formulation of triclopyr was labeled for use in aquatic sites, which allows for basal bark applications when standing water is present. This formulation may have utility for controlling woody plants in standing water. However, anecdotal observations of injury to non-target plants following applications during periods of inundation have been reported. To address this, mesocosm studies were conducted to assess non-target injury through triclopyr root exudation or release from the surface of treated stems via flooding. Mesocosms contained *S. terebinthifolia* as the treated target, while sugarberry (*Celtis laevigata* Willd.), buttonbush (*Cephalanthus occidentalis* L.), and red maple (*Acer rubrum* L.) were included as non-targets. In the first study, the pathway of root exudation for non-target injury following triclopyr (34 g L^{-1}) basal bark application was isolated with activated charcoal placed at the soil surface. In the second study, mesocosms were flooded to assess triclopyr release from the surface of treated stems and subsequent non-target injury. Defoliation of non-target species posttreatment was ≤8%, and triclopyr was detected at ≤5 μg L^{-1} in mesocosm wells when activated charcoal was present. Posttreatment non-target defoliation up to 92%, coupled with triclopyr concentrations in surface waters and wells as high as 4,637 μg L^{-1}, indicated triclopyr movement as a result of flooding. Additionally, triclopyr non-target injury from soil activity independent of flooding was observed. These findings provide limited evidence of triclopyr root exudation but considerable evidence of triclopyr release during flooding following basal bark treatment and support a cautionary approach to basal bark application when standing water is present.

Introduction

Triclopyr is a WSSA Group 4 synthetic auxin herbicide typically used to control woody and herbaceous dicotyledonous plants in range, pasture, and non-crop sites (Shaner 2014; Sisco et al. 1998). Triclopyr stimulates uncontrolled cell division and elongation, leading to abnormal leaf formation, stem swelling, and plant death (Shaner 2014). In natural areas, triclopyr is widely used for invasive herbaceous and woody plant control through individual plant treatment (IPT) techniques (Miller et al. 2015). For woody plant control, triclopyr IPT techniques vary by formulation, primarily due to differences in product solubility in water or oil. The water-soluble triethylamine formulation can be applied as a foliar spray, cut stump, or injection treatment (Anonymous 2016a), while the oil-soluble butoxyethyl ester formulation is commonly applied as a basal bark treatment in an oil carrier (Anonymous 2015).

In 2009, a new triclopyr acid formulation that can be mixed with oil or water was registered for use in ornamental turf, rights of way, and aquatic and non-crop sites (Anonymous 2016b). Given the acid formulation's aquatic site registration and oil solubility, basal bark applications for woody plant control can now be implemented when standing water is present. This was not previously allowed for basal bark application with triclopyr ester, which is only used in uplands and seasonally dry wetlands (Anonymous 2015). The triclopyr acid formulation has been shown to control woody shrubs such as Brazilian peppertree (*Schinus terebinthifolia* Raddi), which is widespread in Florida wetlands (Bell 2019).

Schinus terebinthifolia is an evergreen shrub to small tree that is native to Brazil, Argentina, and Paraguay (Cuda et al. 2006; Mukherjee et al. 2012). This species is invasive to Florida, where it negatively impacts native plant communities by modifying soil structure, nutrient cycling, microbial community composition, and resource availability (Carneiro et al. 1996; Cuda et al. 2006; Dawkins and Esiobu 2016; Doren et al. 1991; Morton 1978). *Schinus terebinthifolia* forms dense stands due to its high capacity for vegetative recruitment, multistemmed structure, and ability to reach 15 m in height (Bell 2019; Cuda et al. 2006; FDACS 2018). The degree of its infestation in Florida alone is estimated at 280,000 ha (Cuda et al. 2006; FDACS 2021) covering mesic habitats, coastal strands, marshes, swamps, wet flatwoods, and mangrove forests of central and southern Florida (FDACS 2021). It ranks as the fourth costliest invader in terms of management in Florida (Hiatt et al. 2019).

While the ability to treat with triclopyr acid in flooded or seasonally wet conditions is useful to applicators, there have been anecdotal reports of non-target injury when it is used for basal bark treatments in south Florida wetlands (J Morton, U.S Army Corp of Engineers, personal communication). Potential affected species include native trees such as sugarberry (*Celtis laevigata* Willd.) and red maple (*Acer rubrum* L.). Pathways of triclopyr movement leading to non-target injury in wetlands, especially following basal bark application, are unknown. In volatility studies, Bauerle et al. (2015) found that triclopyr acid resulted in cotton (*Gossypium hirsutum* L.) and tomato (*Solanum lycopersicum* L.) injury that was not different from the nontreated controls at 14 d after treatment (DAT). If volatility is an unlikely pathway for non-target injury, potential pathways include root exudation, physical release from the surface of treated stems during precipitation or flooding, or herbicide runoff from stems following application.

Previous studies on triclopyr root exudation have focused on the ester formulation in upland settings and reported that root exudation is not a primary pathway for non-target injury (Eck and McGill 2007; Futch and Weingarten 2010; Graziano et al. 2022; Kochenderfer 1999; Willoughby 1999). For example, Graziano et al. (2022) found only one triclopyr detection in soil beneath 20 trees receiving a basal bark treatment of triclopyr ester and reported only 5% non-target injury in nearby vegetation. However, root exudation of the triclopyr acid formulation remains untested, and the potential for triclopyr root exudation in wetland scenarios, where there is the presence of a high water table, is unknown.

Posttreatment flooding is another potential mechanism of off-target movement and subsequent non-target injury. Precipitation and flooding events can lead to herbicide wash-off from treated stems and foliage, contributing to runoff, off-target herbicide deposition, and the accumulation of herbicide residues in soil (Harrington et al. 2016; Holmes and Berry 2009; Norris et al. 1987; Nowak and Ballard 2005; Wilcock et al. 1991). This can increase non-target plant exposure to herbicide (Graziano et al. 2022; Holmes and Berry 2009). Nowak and Ballard (2005) reported a 0.5-m radius of non-target injury during basal bark treatments of triclopyr ester. Holmes and Berry (2009) conducted basal bark treatments with triclopyr ester and attributed the detection of triclopyr soil residues at 91 DAT to winter rains. While there is evidence for non-target injury due to precipitation events after herbicide applications, current research is limited to triclopyr ester in upland habitats.

To address the need for information on the behavior of triclopyr acid in wetland conditions, our objective was to examine both physiological (i.e., root exudation) and physical (i.e., wash-off due to flooding) pathways that may facilitate triclopyr acid non-target injury. Our hypotheses included the following: (1) root exudation would be limited and not a significant factor for non-target injury; and (2) flooding after basal bark treatment would result in significant non-target injury. A better understanding of the pathways of triclopyr non-target injury would improve herbicide stewardship guidelines when conducting basal bark treatments when standing water is present.

Materials and Methods

Two separate greenhouse experiments were conducted at the University of Florida's Center for Aquatic and Invasive Plants (29.72017° N, 82.41563° W) to evaluate the potential pathways of root exudation and wash-off from flooding for off-target triclopyr movement and non-target injury following basal bark treatments to *S. terebinthifolia*. The root exudation experiment was conducted twice, with treatment of the first run on September 14, 2020, and treatment of the second run on June 16, 2022. The flooding experiment was also conducted twice, with treatment of the first run on September 22, 2020, and treatment of the second run on June 15, 2022. Both experiments consisted of basal bark treatments to *S. terebinthifolia* in 94-L mesocosms that also contained *C. laevigata*, buttonbush (*Cephalanthus occidentalis* L.), and *A. rubrum*. These native species provide benefits to wildlife and are impacted by the spread of invasive plants such as *S. terebinthifolia* (Snyder 1991; Sullivan 1993; Swearingen and Bargeron 2016; Tirmenstein 1991).

Plant Propagation

Schinus terebinthifolia was grown from seed collected from West Delray Regional Park (26.45368°N, 80.21839°W) in south Florida. Seedlings were first established in 3.8-L pots (Nursery Supply, Fairless Hills, PA 19030) filled with commercial potting mix (Jolly Gardener Pro Line 44N, Old Castle Lawn and Garden, Atlanta, GA 31146) amended with 0.5 g of slow-release fertilizer (Osmocote© Smart-Release Plant Food, Scotts-Sierra Horticultural Products, Marysville, OH 43040). *Schinus terebinthifolia* saplings were grown for approximately 8 mo before initiation of experiments. *Celtis laevigata*, *C. occidentalis*, and *A. rubrum* saplings were acquired from a local native plant nursery (Urban Forestry, Micanopy, FL 32667) and were approximately 6 mo old at the beginning of the study.

Plants were selected based on uniformity within their species and prepped for transplant by gently washing their roots to remove all organic matter. One individual of each species, including *S. terebinthifolia*, *C. laevigata*, *C. occidentalis*, and *A. rubrum*, was transplanted into each mesocosm. *Schinus terebinthifolia* saplings were transplanted directly into the center of the mesocosm, and native species were planted equidistantly 25 cm from center of each tub (Figure 1A). Plants were acclimated to the mesocosms for 1 additional month before treatment.

Mesocosm Description

Mesocosms were established in a polyethylene plastic greenhouse for Run 1 and a glasshouse for Run 2. Each mesocosm consisted of one 94-L blow-molded high-density polyethylene container (BWI, Nash, TX 75569) filled with pure builder's sand (Vulcan Materials Company Keuka Sand Mine, Melrose, FL 32666) and amended with 1.5 g of the slow-release fertilizer (Osmocote© Smart-Release

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Figure 1. Mesocosm design and treatment approach. (A) A 94-L mesocosm with a polyvinyl chloride (PVC) irrigation well in the back, a PVC sampling well in the front, and the plant spacing of *Schinus terebinthifolia*, *Celtis laevigata*, *Cephalanthus occidentalis*, and *Acer rubrum*. (B) A longitudinal section of a mesocosm with a 5-cm layer of powdered activated charcoal capped with 2.5 cm of builder's sand. Note the prolific reddish-colored roots of *S. terebinthifolia*. (C) A basal bark application using a 10-ml micropipette to deliver 5 ml of each treatment solution to the lower 30 cm of each *S. terebinthifolia* stem.

Plant Food). Each mesocosm contained two 5-cm-diameter polyvinyl chloride (PVC) wells, one for subirrigation and the other on the opposite side for water sampling (Figure 1A). The sampling well had 24 drilled holes (10-mm diameter) evenly spaced around the lower 20 cm and covered with a mesh screen to exclude sand from the well. Subirrigation was applied daily throughout the experiments to maintain a perched water table in each mesocosm, 20 cm from the base of the tubs and 20 cm from the soil surface.

Root Exudation Study

Experiments were a completely randomized design with four replications. Four basal bark treatments were tested: (1) triclopyr acid applied at 34 g L^{-1} (Trycera®, Helena Agri-Enterprises, Collierville, TN 38017) in an oil carrier (Impel Red Oil®, Helena Agri-Enterprises); (2) triclopyr acid (34 g L^{-1}) applied in an oil carrier with a layer of activated charcoal added at the surface of each mesocosm (Biogize SD Soil Detox™, Garden Variety Organics, Waxahachie, TX 75165); (3) basal bark oil applied with no herbicide; and (4) basal bark oil applied with no herbicide with a layer of activated charcoal at the surface of each mesocosm. For treatments requiring activated charcoal, a 5-cm layer of powdered activated charcoal was spread evenly across the soil surface of mesocosms and capped with 2.5 cm of pure builder's sand 1 d before basal bark applications (Figure 1B). The removal of all organic matter from plant roots, coupled with the use of pure sand as the growing medium and the surface layer of activated charcoal, were intended to isolate triclopyr non-target injury strictly through the pathway of root exudation.

Basal bark applications were made with a 10-ml micropipette (Mettler Toledo, Columbus, OH 43240) set to deliver 5 ml to the single *S. terebinthifolia* shrub in each mesocosm (Figure 1C). Applications were carefully made to the full circumference of each *S. terebinthifolia* stem from the soil surface to a height of 30 cm and were comparable to typical field applications approximating 5 ml of herbicide–oil mix per inch of stem diameter. Applications were made from top to bottom to allow gravity to facilitate stem coverage without significant runoff (Figure 1C). The micropipette approach ensured complete stem coverage and protected the non-target species from directly receiving any herbicide from drift or accidental direct application that can occur with typical pressurized basal bark sprayers. Additionally, the wells were capped to prevent direct herbicide contamination during the application process.

Flooding Study

This experiment was set up as a completely randomized design with four replications. Four basal bark treatments were tested: (1) triclopyr acid (34 g L^{-1}) applied in an oil carrier; (2) triclopyr acid (34 g L^{-1}) applied in an oil carrier followed by postapplication flooding; (3) basal bark oil applied with no herbicide; and (4) basal bark oil with no herbicide followed by postapplication flooding. Basal bark herbicide treatments were applied as described for the root exudation study.

For the flooding treatments, water was applied through the subirrigation well immediately after basal bark treatments were completed. Plants were flooded to a depth of 7.5 cm above the soil surface (Figure 2A). This resulted in 25% of the total basal bark–treated stem area being submersed. The use of the subirrigation wells allowed flooding to occur without physically washing the oil and oil herbicide mixture from treated stems. Flooding was maintained daily at a depth of 7.5 cm above the soil surface for 21 DAT, at which time water levels were allowed to naturally recede into the sand through evaporation and evapotranspiration. Following 21 DAT, flooded mesocosms were watered in the same manner as nonflooded mesocosms to maintain a perched water table of 20 cm relative to the base of the tub.

Data Collection

For both studies, non-target injury data were collected as visual estimates of percent canopy defoliation at 49 DAT. Defoliation was

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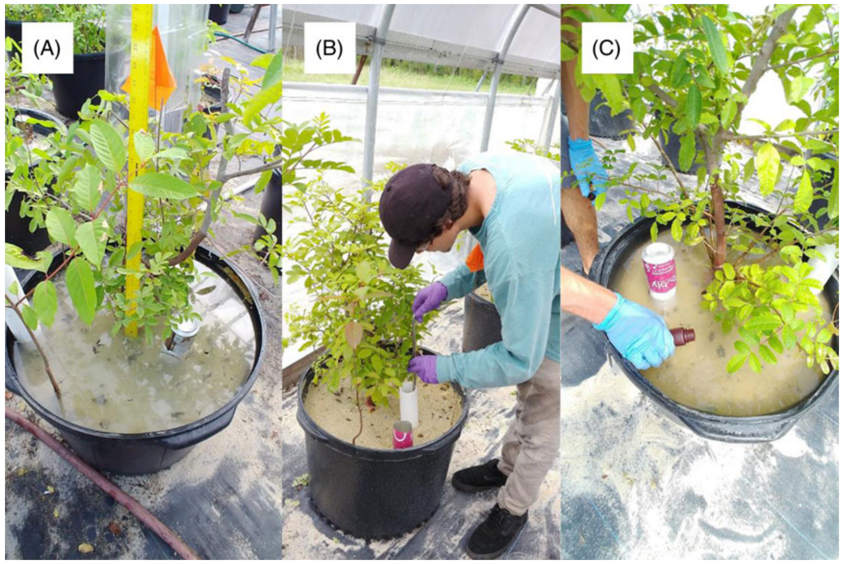

Figure 2. Triclopyr flooding study mesocosm design and water sampling approach. (A) A 94-L mesocosm that was flooded to a depth of 7.5 cm above the soil surface immediately following basal bark application. (B) Collection of a water sample from the sample well of a mesocosm. (C) Collection of surface-water samples from a flooded mesocosm. Aluminum cans were used to cover sample wells when they were not in use.

Table 1. Target (*Schinus terebinthifolia*) and non-target (*Celtis laevigata*, *Cephalanthus occidentalis*, and *Acer rubrum*) defoliation (mean ± SE) at 49 days after basal bark treatment.

Basal bark treatment	Activated charcoal layer[a]	*S. terebinthifolia*	*C. laevigata*	*C. occidentalis*	*A. rubrum*
		% Defoliation[b]			
Oil carrier only	Absent	6 ± 2 b	7 ± 3 b	2 ± 0 c	2 ± 1 b
Oil carrier only	Present	5 ± 2 b	6 ± 2 b	2 ± 0 c	1 ± 0 b
Oil carrier + triclopyr (34 g ae L^{-1})	Absent	100 ± 0 a	62 ± 12 a	11 ± 2 a	24 ± 11 a
Oil carrier + triclopyr (34 g ae L^{-1})	Present	99 ± 1 a	8 ± 2 b	7 ± 1 b	5 ± 1 b

[a]Activated charcoal was used to isolate triclopyr root exudation as a pathway for non-target damage. It was placed in a 5-cm layer at the surface and capped with 2.5 cm of sand.
[b]Means within columns followed by the same letter are not different according to Fisher's protected LSD test (α = 0.05).

estimated for each plant species and was based on a scale of 0% to 100%, where 0% indicated no loss of foliage and 100% indicated a complete loss of foliage. Water sampling was conducted weekly during that period for both studies, with additional sampling at 1 and 3 DAT for the flooding study. Water sampling was not conducted at time zero for the root exudation study, as triclopyr concentration at that time was assumed to be zero. Initial water sampling was delayed 24 h after treatment for the flooding study based on the assumption that triclopyr values would be negligible at the time of flooding. Water was sampled from the PVC sampling well of each mesocosm. Water levels were measured before sampling and adjusted to a consistent water depth of 20 cm, relative to the base of each mesocosm. Water samples were collected with a 100-ml stainless steel dipper (Supply My Lab, Swedesboro, NJ 08085) and transferred to a 50-ml vial (Figure 2B). The stainless steel dipper was thoroughly cleaned between sampling of each mesocosm by washing it with dish detergent and/or acetone if needed. All vials were placed on ice immediately upon collection and preserved in a freezer at −20 C for 3 to 6 mo until subject to analysis. In the flooding study, surface-water samples were also collected weekly until 21 DAT following the same protocol (Figure 2C). Wells were capped throughout the duration of the study and only opened for watering or water sample collection.

Triclopyr Quantification

Triclopyr concentrations in water samples from the first runs of both studies were quantified using a modified solid-phase extraction (SPE) method described by Werner et al. (1996). Water samples were raised to room temperature (21 C), analyzed for pH, and fortified with 400 mg of sodium chloride. SPE cartridges (ENVI-Carb™ SPE Tube 57094, MilliporeSigma 28820 Single Oak Drive, Temecula, CA 92590) were conditioned by passing 5 ml of methylene chloride:methanol (80:20 v/v) twice, 5 ml of methanol (100% by volume) once, followed by three 5-ml additions of ascorbic acid solution (10% by volume) using a vacuum pump and manifold. Water samples (50 ml) were passed through the conditioned SPE cartridges under vacuum. The SPE cartridges were then air-dried by maintaining them under vacuum for 30 min. Triclopyr acid was eluted from the columns into glass vials using 1 ml of methanol (100% by volume), followed by 4 ml methylene chloride: methanol (80:20 v/v) plus 0.1% formic acid. The eluate was concentrated to 1 ml in a heated water bath and transferred to a 2-ml glass vial for analysis.

Samples for Run 2 of both studies were analyzed using a novel direct-injection method, which provided a more efficient means of processing samples through a 60% reduction in labor. Sample preparation for this method involved filtering 5 ml of each water

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sample and transferring 1 ml of filtered samples into 2 ml glass vials. Fifty microliters of formic acid was added to each 2-ml glass vial, concluding sample preparation.

All samples were analyzed using an Agilent 1290 ultra-high performance liquid chromatography (UHPLC) system coupled with an Agilent 6495C tandem mass spectrometer. The UHPLC was equipped with a Zorbax Eclipse Plus C18 Rapid Resolution HD column (2.1 by 100 mm, 1.8 micron) and used two mobile phases (mobile phase A = 5 mmol ammonium formate plus 0.1% formic acid in optima water; mobile phase B = 5 mmol ammonium formate plus 0.1% formic acid in methanol) at a flow rate of 0.4 ml min^{-1} (UHPLC and Zorbax, Agilent Technologies, Santa Clara, CA 95051). The gradient changed from 95% A to 0% A in 4.5 min, with a 1-min hold at 0% A. The column was then preconditioned at 95% A for 2 min before the next run. Triclopyr was measured in multiple reaction monitoring, positive ionization mode at m/z 255.9 as the parent molecule, m/z 195.9 as the qualifier, and m/z 197.9 as the quantifier. Triclopyr had a retention time of 4.486 min. All concentrations were quantified using external standard calibration curves (Triclopyr, Pestanal®, analytical standard, MilliporeSigma, 400 Summit Drive, Burlington, MA 01803). Percent recoveries from spiked samples were greater than 88% in all studies. Quality assurance/quality control included instrument blanks, sample blanks, sample spikes, and sample spike duplicates. The minimum method quantification limit (MQL) was 0.482 and 5.0 μg L^{-1} for Run 1 and Run 2, respectively. Water samples that fell below the direct-injection MQL were subsequently reanalyzed through extraction by using the modified SPE method described previously.

Statistical Analysis

Statistical analyses were conducted in RStudio (RStudio 1.4.1717). All data were subjected to mixed-model ANOVA in which treatment was considered a fixed effect and experimental run was considered a random effect (Blouin et al. 2011). Data were arcsine square-root transformed to meet assumptions and back-transformed for presentation (Snedecor and Cochran 1967). Means were separated using Fisher's protected LSD test ($\alpha = 0.05$) in the AGRICOLAE package (v. 1.3-5; Felipe 2021). Figures were generated using the GGPLOT2 package (v. 3.4.0; Wickham 2016).

Results and Discussion

Root Exudation Study

In this study, we investigated the potential for root exudation of triclopyr. We hypothesized that root exudation of triclopyr acid would be limited and not a significant factor for non-target injury. This was generally supported by our results.

Schinus terebinthifolia defoliation was 99% or greater at 49 DAT for the triclopyr plus oil treatment and the triclopyr plus oil plus activated charcoal treatment (Table 1). Triclopyr efficacy was not limited by the presence of the activated charcoal layer, which indicates sufficient triclopyr penetration occurred through the outer bark to the phloem and cambium. Basal oil applied without triclopyr resulted in approximately 6% *S. terebinthifolia* defoliation, and this was not different when activated charcoal was present.

Celtis laevigata percent defoliation was 62% in the triclopyr plus oil treatment when there was no activated charcoal present (Table 1). However, when activated charcoal was present in triclopyr-treated mesocosms, *C. laevigata* defoliation was 8% and was not different from defoliation observed in the control treatments without triclopyr. These findings indicate significant injury occurred from triclopyr moving directly into the sand during the application but not through root exudation.

For *C. occidentalis*, defoliation was 11% in the triclopyr plus oil treatment, and this was greater than in all other treatments (Table 1). The triclopyr plus oil plus activated charcoal treatment resulted in 7% defoliation, and this was significantly higher than the 2% defoliation observed in both control treatments without triclopyr. Although minor, this 5% increase in defoliation between these treatments was the only occurrence of injury that could be attributed to triclopyr root exudation.

Acer rubrum exhibited 24% defoliation in the triclopyr plus oil treatment. This was significantly greater than defoliation in all other treatments (Table 1). *Acer rubrum* defoliation was 5% in the triclopyr plus oil plus activated charcoal, and this was not different from defoliation observed in the control treatments without triclopyr. Similar to *C. laevigata* data, these findings indicate significant injury occurred from triclopyr moving directly into the sand during the application but not through root exudation.

For the triclopyr plus oil treatment, triclopyr concentrations in sampling wells increased over time and reached 487 μg L^{-1} at 42 DAT (Figure 3). This was in contrast to the triclopyr plus oil plus activated charcoal treatment, where triclopyr concentrations in sampling wells were less than 5 μg L^{-1} across all sampling times. These results provide support for triclopyr soil activity as a driver of defoliation for all three non-target species. Beyond the limited defoliation *C. occidentalis* experienced in the triclopyr plus oil plus activated charcoal treatment, these results do not support root exudation as a pathway for significant off-target triclopyr movement and associated non-target injury.

This experiment provides insight into the low likelihood of triclopyr root exudation and its role as a pathway for non-target injury when triclopyr acid was applied as a basal bark treatment in mesocosms designed to simulate wetland conditions. We detected triclopyr concentrations of less than 5 μg L^{-1} at any sample date when activated charcoal was present, and we observed only a minor increase in defoliation of *C. occidentalis* compared with the nontreated controls when triclopyr was applied with activated charcoal present.

Although we only tested the triclopyr acid formulation, results are in agreement with previous investigations of root exudation of

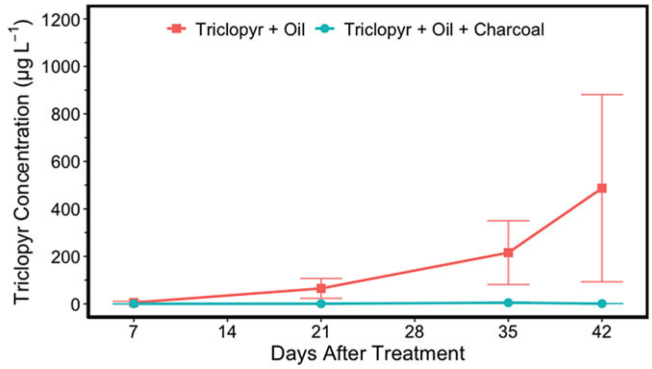

Figure 3. Triclopyr concentration (μg L^{-1}) detected in mesocosm wells at 7, 21, 35, and 42 d after treatment in the triclopyr root exudation study. Triclopyr concentrations were derived only for treatments that included basal bark oil applications with triclopyr at 34 g L^{-1} with and without a 5-cm layer of powdered activated charcoal placed at the surface of the mesocosm. Error bars represent 1 standard error of the mean ($N = 8$).

Table 2. Target (*Schinus terebinthifolia*) and non-target (*Celtis laevigata*, *Cephalanthus occidentalis*, and *Acer rubrum*) defoliation (mean ± standard error) at 49 days after basal bark treatment.

Basal bark treatment	Flooding treatment[a]	*S. terebinthifolia*	*C. laevigata*	*C. occidentalis*	*A. rubrum*
		% Defoliation[b]			
Oil carrier only	Nonflooded	6 ± 2 c	16 ± 3 c	4 ± 2 b	4 ± 2 bc
Oil carrier only	Flooded	19 ± 5 b	22 ± 9 c	9 ± 3 b	3 ± 1 c
Oil carrier + triclopyr (34 g ae L^{-1})	Nonflooded	100 ± 0 a	51 ± 11 b	39 ± 11 a	30 ± 13 b
Oil carrier + triclopyr (34 g ae L^{-1})	Flooded	100 ± 0 a	92 ± 5 a	52 ± 14 a	69 ± 14 a

[a]Flooding treatments were applied to determine the contribution of triclopyr release from floodwaters as a pathway for non-target injury. Mesocosms were flooded immediately after basal bark treatment to a depth of 7.5 cm above the soil surface for 21 d.
[b]Means within columns followed by the same letter are not different according to Fisher's protected LSD test (α = 0.05).

the butoxyethyl ester formulation. These concluded it is limited and unlikely to result in non-target injury (Eck and McGill 2007; Graziano et al. 2022; Harrington et al. 2016; Kochenderfer 1999). Additionally, although not part of this study, the transfer of herbicides from target plants to non-target vegetation through interspecific root grafting is also rare and has not been observed with triclopyr (Eck and McGill 2007; Futch and Weingarten 2010; Kochenderfer et al. 2006; Willoughby 1999).

These results suggest that in a basal bark treatment of a single tree, triclopyr released by root exudation has a low potential for contributing to unrecoverable non-target injury. We recommend the use of triclopyr for basal bark treatments when non-target injury must be avoided. However, land managers should anticipate greater triclopyr release and an elevated risk for non-target injury when performing basal bark treatments in high-density plant infestations, due to increased herbicide output (Graziano et al. 2022). We recommend that future research should focus on investigating alternative mechanisms of non-target injury that remain untested for triclopyr, as well as approaches that seek to improve triclopyr herbicide stewardship in basal bark treatments.

Flooding Study

We hypothesized that non-target injury would be greater when flooding occurred after basal bark treatment with triclopyr acid compared with nonflooded treatments; this was strongly supported by our results, which demonstrated high non-target injury and the presence of triclopyr in the surface and well water when flooding occurred after basal bark treatment.

Schinus terebinthifolia percent defoliation was 100% at 49 DAT for triclopyr treatment with and without flooding (Table 2). Application of basal oil only and subsequent flooding resulted in 19% defoliation for *S. terebinthifolia*, which was greater than defoliation observed in the oil only treatment with no flooding. In that treatment, defoliation was approximately 6%. This indicated *S. terebinthifolia* exhibited some stress in response to flooding alone, as evidenced by the loss of leaves.

Celtis laevigata defoliation was 92% in the triclopyr plus oil plus flooding treatment and was 41% greater than in the triclopyr plus oil without flooding treatment (Table 2). These values were both greater than defoliation in flooded and nonflooded mesocosms not treated with triclopyr. *Cephalanthus occidentalis* defoliation was 52% in the triclopyr plus oil plus flooding treatment. This was not different from defoliation in the triclopyr plus oil without flooding treatment. These were both greater than defoliation in flooded and nonflooded mesocosms not treated with triclopyr.

Across treatments *A. rubrum* followed a defoliation pattern similar to *C. laevigata*. Defoliation was 69% in the triclopyr plus oil

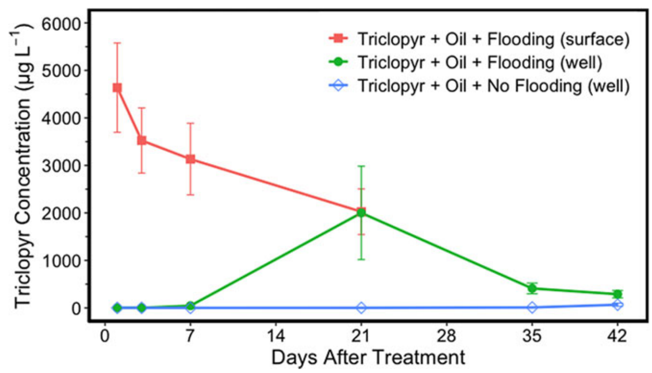

Figure 4. Triclopyr concentration (µg L^{-1}) detected in mesocosm wells at 1, 3, 7, 21, 35, and 42 d after treatment in the triclopyr flooding study. Triclopyr concentrations for surface water could only be collected through 21 d after flooding, as the water level receded before the next sample date. Triclopyr concentrations were derived only for treatments that included basal bark oil applications with triclopyr at 34 g L^{-1} with and without flooding. Error bars represent 1 standard error of the mean ($N = 8$).

plus flooding treatment and was 39% greater than defoliation in the triclopyr plus oil without flooding treatment (Table 2). These values were both greater than defoliation in flooded and nonflooded mesocosms not treated with triclopyr.

Triclopyr concentrations were highest in surface water of triclopyr-treated flooded mesocosms, measuring 4,637 µg L^{-1} at 1 DAT and 2,025 µg L^{-1} at 21 DAT (Figure 4). Likewise, triclopyr concentrations in well samples of flooded mesocosms averaged 2,000 and 288 µg L^{-1} at 21 and 42 DAT, respectively. The lowest triclopyr concentrations detected were consistently found in sampling wells of nonflooded, triclopyr-treated mesocosms and averaged 1.5 and 67 µg L^{-1} at 21 and 42 DAT. These data strongly support high non-target injury potential when flooding occurs after basal bark treatment with triclopyr acid.

This experiment expands knowledge on an additional pathway of non-target injury associated with basal bark applications of triclopyr in wetlands. Our hypothesis that non-target injury would increase if flooding occurred directly after basal bark treatment with triclopyr acid was strongly supported by our results. This experiment demonstrated significantly higher injury to two of the three non-target species in flooded versus nonflooded conditions and triclopyr concentrations as high as 4,637 µg L^{-1} in the water when flooding occurred. In addition, our results also provide greater insight into the response of *S. terebinthifolia* to prolonged inundation in freshwater, where we observed stress from flooding independent of herbicide treatment.

Although it was not statistically compared, we also observed some variation in the nontarget species response to these

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triclopyr concentrations. Existing literature supports variation in interspecies susceptibility to triclopyr and, to a lesser degree, variation in intraspecies susceptibility to differing triclopyr formulations (de Mendiburu 2021; Forster et al. 1997; Hutchinson and Langeland 2010; Kochenderfer 1999; Self 2020). Based upon this work, we suggest that additional research is needed to evaluate potential interspecies variation in susceptibility to the triclopyr acid formulation. Further investigation would increase the collective understanding of how common native species respond to triclopyr acid and could be used to improve triclopyr herbicide stewardship.

Previous studies have also attributed the detection of triclopyr residues in the vicinity of treated vegetation from wash-off that resulted from precipitation and or flooding events and support our findings (Harrington et al. 2016; Holmes and Berry 2009; Wilcock et al. 1991). Our study simulated a flooding event immediately after treatment, but given our results and existing literature support, we suggest that land managers proceed cautiously with basal bark applications in areas prone to flooding and recommend additional research to further assess the impacts of flooding after treatment in field conditions. Direct basal bark application to the base of the trunk is also not practical in standing-water conditions, leaving managers unsure on how to best proceed. As such, greater research that examines altering the basal bark application technique to offset these flood-related challenges, such as using high band treatments, may also benefit land managers. The results of these studies provide greater insight into the pathways of triclopyr acid non-target injury and can be used by land managers and applicators to improve triclopyr herbicide stewardship.

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