THESIS

ENDOTHALL BEHAVIOR IN FIVE AQUATIC WEEDS

Submitted by

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ABSTRACT

ENDOTHALL BEHAVIOR IN FIVE AQUATIC WEEDS

Endothall is one of the original aquatic herbicides being primarily to control submersed plants since 1960. Endothall is considered a contact herbicide, in a chemical class of its own, it is a serine/threonine protein phosphatase inhibitor, which has broad-spectrum control and is effective in controlling both monocotyledons and dicotyledons. Eurasian watermilfoil (*Myriophyllum spicatum* L.) (EWM), hydrilla [*Hydrilla verticillata* (L.f.) Royle], curlyleaf pondweed (*Potamogeton crispus* L.) (CLP), and sago pondweed (*Potamogeton pectinatus* L.) (SPW) are submersed aquatic species considered troublesome throughout the United States, which can be controlled with endothall. These species can form extensive, undesirable surface canopies, which can negatively impact water flow, water quality, economic and ecological value of water bodies.

Although endothall is considered a contact herbicide, many field observations suggest that it might have systemic activity. The goals of this research were to (1) determine maximum herbicide absorption and absorption rate, (2) evaluate herbicide translocation from shoots to roots in EWM, two hydrilla biotypes, CLP, and SPW, and (3) evaluate herbicide desorption in EWM and two hydrilla biotypes.

Each weed species was clonally propagated from apical shoot cutting or turions/tubers when present. For herbicide absorption and translocation, plants of each species with developed roots and 15 cm of shoot growth were transferred to test tubes containing unwashed silica sand and sealed at the top with a low melting point eicosane wax to isolate the root system from the water column. Plants were exposed to the herbicide over 192 h. At predetermined time points three plants of each species were harvested, divided into shoot and root tissue, and oxidized. Herbicide desorption was evaluated over 96 h.

Endothall absorption was linear in hydrilla, while in EWM, CLP and SPW it best fit an asymptotic rise function. Translocation to EWM, CLP, and SPW roots was limited, reaching a maximum translocation of 8%, 3% and 1% of total absorbed radioactivity, respectively. Monoecious and dioecious hydrilla showed a linear increase without reaching maximum absorption or translocation 192 HAT. Endothall translocation to monoecious and dioecious hydrilla roots was 18% and 16% of total absorbed radioactivity, respectively. Herbicide desorption was less than 30% for all the three species evaluated. These data provide strong evidence that endothall behaves as a systemic herbicide in these aquatic species.

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Native aquatic plants play an important role in aquatic systems because they provide valuable fish and wildlife habitat, improve water quality and clarity by stabilizing sediments, in addition to reducing rates of shoreline erosion (Savino and Stein 1982; Heitmeyer and Vohs Jr 1984). Water quality improvements occur due to plant absorption of excess nutrients and some water pollutants (Smart et al. 1998). On the other hand, invasive aquatic plants can negatively affect entire aquatic ecosystems and impact many human activities such as water distribution, navigation, and recreation (Netherland et al. 2000; Bowes et al. 1979). Early efforts to management aquatic vegetation were limited to cultural and biological control and mechanical removal; however, since 2,4-D's commercialization in the late 1940s, chemical control has become a common and cost-effective method for selective management of invasive aquatic plants.

Chemical aquatic weed control can range in scale from a backpack sprayer used to treat localized problems and individual plants up to large-scale treatments targeting entire lakes using boats or helicopters. To achieve expected aquatic weed control, the herbicide must remain in the treated area for a certain amount of time, ranging from a few hours up to several months, so the plants are exposed to the lethal herbicide concentration for a sufficient amount of time (Gettys 2014). There are two factors to take into consideration when applying aquatic herbicides, (1) the herbicide concentration in the water column in the treated area, and (2) the length of time the target species are exposed to the lethal herbicide concentration. These two factors have been defined as the concentration and exposure time (CET) relationship, and it is different for each herbicide and plant species.

Contact herbicides are faster acting and require a shorter contact time to achieve control, but systemic herbicides are often preferred because they move within the plant and kill belowground plant parts such as roots and rhizomes, which reduces or eliminates regrowth (Gettys 2014). Contact herbicides are often used in areas with high water exchange, where it is difficult to maintain the required concentration in the water surrounding the target plant, and also in spot treatments in larger water bodies. Systemic herbicides, which are slower acting and usually require longer contact time, are often used in areas with slower water exchange or fully contained systems.

The first inorganic herbicide registered for aquatic use was copper sulfate in the 1950s. Copper is a micronutrient used as a fungicide in agricultural systems and it has been used for aquatic plant control since the early 1900s, even though it was not registered for aquatic use (Gettys et al. 2014). Since copper sulfate was registered, and until the early 2000s, only six other herbicides have been registered for aquatic use (2,4-D, endothall, diquat, acrolein, glyphosate, and fluridone). Working cooperatively with EPA, Dr. William Haller (University of Florida) conducted a massive screening program to identify new candidate herbicides that could be used to manage aquatic plants. This effort resulted in the registration of eight new active ingredients for aquatic use since 2000 (triclopyr, imazapyr, carfentrazone, penoxsulam, imazamox, flumioxazin, bispyribac-sodium, and topramezone) (Gettys et al. 2014) (Table 1).

Endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid) is one of the original aquatic herbicides used primarily to control submersed plants, but initially it was introduced in the 1950s as a selective, post-emergence herbicide for annual broadleaf and grass control in sugar beets. Endothall was also used as a preharvest desiccant in potatoes, alfalfa, and clover seed crops (Shaner 2014). It wasn’t until 1960 that endothall’s label was expanded to include aquatic weed control in static water bodies and in 2010 the label was further expanded for use in flowing water systems (Hiltibran 1962; EPA 2010). Under both aquatic and terrestrial conditions, visual symptoms are similar to those of cold injury, with discoloration, defoliation, and tissue desiccation which results in plant death (MacDonald et al. 1993). Peak injury symptoms usually occur within 4 to 6 weeks after initial treatment (Sprecher et al. 2002).

Despite being a labeled terrestrial herbicide since the 1950s and as an aquatic herbicide since the 1960s, endothall's mode of action was not determined until 2011. Endothall is considered a contact herbicide in its own chemical class (Madsen 1997; Shaner 2014). Endothall is a serine/threonine protein phosphatase (PP) inhibitor (Tresch et al. 2011; Bajsa et al. 2012), which has broad-spectrum control and is effective in controlling both monocotyledons and dicotyledons (Madsen 1997; Westerdahl and Getsinger 1988). Endothall is a close analog of cantharidin, a natural compound produced by the blister beetle (*Epicauta* spp.) and the Spanish fly (*Lytta vesicatoria*) that causes burning and blistering of the skin in humans (MacDonald et al. 1993; Bajsa et al. 2011). Cantharidin is also a strong serine/threonine PP inhibitor, a broad class of PPs that control a large number of signaling processes in plants, and their inhibition disrupts many cellular processes, leading to plant death (Bajsa et al. 2011). Endothall degrades rapidly in both soil and water and has a half-life ranging from 1 to 7 d (Langeland and Warner 1986).

For use in aquatic systems, the free organic diacid endothall is available in two salt formulations, endothall dipotassium salt and endothall mono(N,N-dimethylalkalamine) salt (Figure 1.1). The amine formulation is generally two to three times more active than the dipotassium formulation on algae and macrophytes, but it is also 200 to 400 times more toxic to nontarget aquatic organisms, such as fish, and it is also more persistent in aquatic environment (MacDonald et al. 1993; Sprecher et al. 2002). Endothall has been widely and effectively used to control Eurasian watermilfoil (*Myriophyllum* s*picatum* L.) (EWM), hydrilla [*Hydrilla verticillata* (L.f.) Royle], curlyleaf pondweed (*Potamogeton crispus* L.) (CLP), and sago pondweed (*Potamogeton pectinatus* L.) (SPW) throughout the United States (Skogerboe and Getsinger 2001, 2002). These four aquatic weeds are the main focus of the studies conducted between 2016 and 2017 at Colorado State University presented in the next two chapters.

Two of the most commonly occurring submersed invasive species in the US are EWM and hydrilla. These plants can form extensive undesirable canopies which can negatively affect water quality and native plant communities by limiting light penetration, significantly reducing dissolved oxygen, increasing water temperature, and impacting recreational uses such as swimming, fishing, and boating. (Netherland et al. 2000; Bowes et al. 1979).

EWM is native to Eurasia and it was introduced in the United States in the 1940s as an ornamental plant for aquariums (Couch and Nelson 1985). It is one of the most costly aquatic plants to manage (Pimentel 2009). After its introduction, this submersed aquatic macrophyte spread rapidly throughout the US, and it is now present in at least 49 states (USGS 2018b). EWM is a dicotyledonous, herbaceous perennial plant. It produces viable seeds, but its invasiveness is mainly due to autofragmentation (Smith and Barko 1990). New shoots grow towards the water surface early in the growing season, where it flowers once it reaches the surface and then fragments. Autofragmentation usually occurs soon after flowering and new living fragments often fall to the bottom and form new plants (Vassios et al. 2011). One single node is enough to start a new plant (Grace and Wetzel 1978).

EWM is most commonly found in waters 1 to 4 m deep. It can also grow in water 10 m deep, but as the shoots grow, lower leaves drop off in response to low light (Smith and Barko 1990). EWM has a relatively high optimum temperature of 32 C, but can grow over a wide range of temperatures from as low as 10 C to as high as 38 C (Smith and Barko 1990). This ability to grow in low temperatures allows it to establish early in the growing season, and compete effectively with native species (Barko et al. 1982).

Hydrilla is an aggressive submersed weed and is one of the most difficult invasive aquatic weeds to manage in the United States. It has now been reported in at least 27 states (USGS 2018a). Monoecious and dioecious hydrilla biotypes can be found in the US. The dioecious biotype is more commonly found in the southern US, and was the first biotype to be introduced in the country, while the monoecious biotype is more commonly found from North Carolina northward. The monoecious biotype is the more cold-tolerant of the two biotypes (Dayan and Netherland 2005; True-Meadows et al. 2016).

Hydrilla is a monocotyledonous herbaceous perennial species and like EWM, it can reproduce through plant fragments. Only the female form of dioecious hydrilla has been reported in the US, preventing sexual reproduction; therefore, dioecious hydrilla can only spread by vegetative means in the US as it does not produce seeds. In addition to vegetative fragments, hydrilla also produces tubers and turions. Tubers and turions are both overwintering propagules, but morphologically distinct structures. Turions grow in the leaf axils and detach upon maturity. They are small, dark green structures, while tubers are produced on the terminal end of the rhizome and they are larger, yellowish structures (True-Meadows et al. 2016). These underground tubers can remain dormant for several years and can survive for several days out of water and up to 5 years in moist sediments. In a single year, a sprouting tuber planted in shallow water can produce over 200 tubers per square foot (Gettys et al. 2014). Hydrilla is also uniquely adapted to grow at lower light levels, which allows it to grow in deeper water than most native submersed species.

CLP was first identified in Delaware in 1859 (Stuckey 1979) and is also a submersed, aquatic macrophyte, native to Europe, Asia, Africa, and Australia. It is an herbaceous perennial, monocotyledonous plant that senesces in the summer. Although it is a perennial species, it has characteristics of an annual species as it senesces completely in early summer and only the turions and seeds "over-summers" (Netherland et al. 2000). This species was named after its wavy leaf structure, making it very easy to identify. CLP can thrive in a wide range of growing conditions, from summer conditions with very warm temperature to ice-covered water with very low light intensities (Gettys et al. 2014). CLP is considered a cool water plant with a unique life cycle for submersed aquatic plants.

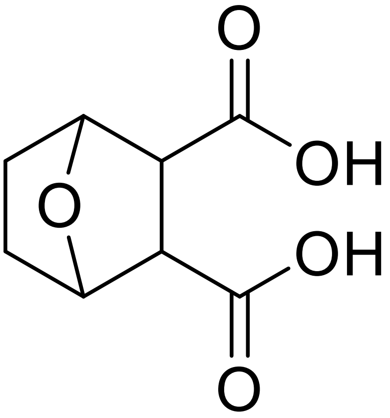
It reproduces primarily by producing turions (hardened modified reproductive buds that form from apical buds and leaf axils), but it also produces rhizomes and viable seeds. Although CLP seed production can be prolific, less than 0.1% could be stimulated to germinate. CLP turions can remain dormant for several years, which makes them extremely difficult to manage (Barr and DiTomaso 2014). Plants achieve their maximum density in late spring, which is when they flower and produce turions, then the plant senesce in early summer. Turions are dormant during the summer, and sprout in the fall when daylength shortens, and water temperatures drop. Early season herbicide treatments can control CLP effectively and prevent turion formation (Poovey et al. 2002).

SPW is a native submersed macrophyte that can be found worldwide, but occurs most often in temperate regions. In these climates, SPW is one of the first species to grow in the spring, giving it an advantage when compared to other native species that start actively growing a few months later (Kantrud 1990). SPW is a key species for most wetland/riparian restoration projects because it is an important food source for many waterfowl. SPW becomes a major problem in flowing water systems, such as irrigation canals and drainage channels. Once established, the plant forms dense, monotypic stands that can choke irrigation canals and significantly reduce water flow. SPW spreads primarily through extensive rhizomes, but it persists by the production of subterranean tubers (Slade et al. 2008). A single plant can produce tens of thousands of tubers in one growing season, making it a very prolific colonizer (Yeo 1965).

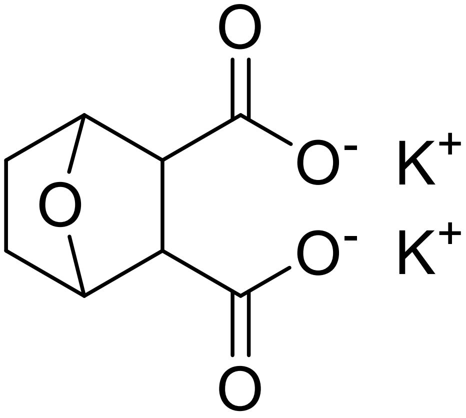
In conclusion, there are approximately 225 herbicides registered in the United States, but only 14 are currently registered for use in aquatic systems (Table 1.1), which places aquatic plant management at a significant disadvantage. Although the discovery of herbicide resistance in hydrilla (Michel et al. 2004) was an important driving force for the registration of new herbicides, there are still far fewer tools for aquatic plant management. Endothall, a broad-spectrum herbicide, controls most of the undesired aquatic plants, including the five species described before and discussed in the next chapters.

Table 1.1: Registered aquatic herbicides, year of registration, primary use pattern, and mode of action.

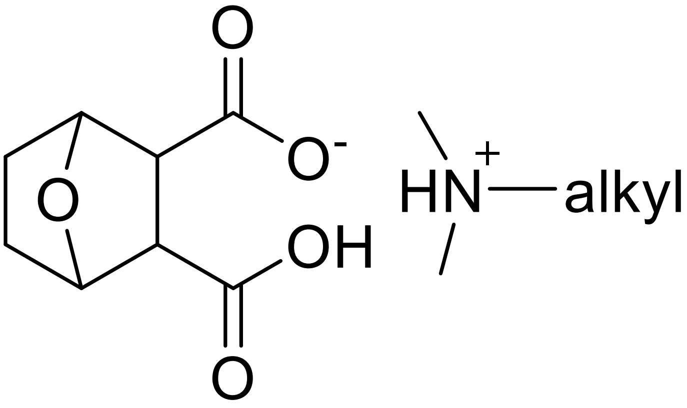
Herbicide	**Year of Registration**	**Primary use**				**Mode of Action**
		Submersed	**Floating**	**Emergent**	**Algae**	
Copper	1950s	X	X		X	Contact; Plant cell toxicant
2,4-D	1959 (ester) 1976 (amine)	X	X	X		Systemic; Plant growth regulator
Endothall	1960	X	X		X	Contact; Protein phosphatase inhibitor
Diquat	1962	X	X	X		Contact; PSI inhibitor
Acrolein	1965	X	X		X	Contact; Plant cell toxicant
Glyphosate	1977			X		Systemic; EPSPS inhibitor
Fluridone	1986	X	X			Systemic; PDS inhibitor
Triclopyr	2002	X	X	X		Systemic; Plant growth regulator
Imazapyr	2003			X		Systemic; ALS inhibitor
Carfentrazone	2004	X	X	X		Contact; PPO inhibitor
Penoxsulam	2007	X	X			Systemic; ALS inhibitor
Imazamox	2008	X	X	X		Systemic; ALS inhibitor
Flumioxazin	2011	X	X	X		Contact; PPO inhibitor
Bispyribac	2012	X	X			Systemic; ALS inhibitor
Topramezone	2013	X	X			Systemic; HPPD inhibitor



Endothall diacid



Endothall dipotassium salt



Endothall mono(N,N-dimethylalkylamine) salt

Figure 1.1: Chemical structure of endothall and its salts.

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Chapter 2: Endothall Absorption, Translocation, and Desorption by Eurasian Watermilfoil and Hydrilla

INTRODUCTION

Aquatic invasive plants can negatively affect entire aquatic systems and herbicides are one of the most important management options for their control. Endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid) was commercialized in the 1950s as a selective, post-emergence herbicide to control annual broadleaf and grassy weeds in sugar beet and also as a pre-harvest desiccant in potato, alfalfa, and clover seed crops (Shaner 2014). Endothall's use as an aquatic herbicide began in the 1960s with two commercial products: mono(N,N-dimethylalkylamine) salt (Hydrothol® 191) and dipotassium salt (Aquathol® K). The dimethylalkylamine salt was used primarily to control algae, while the dipotassium salt was used to control submersed monocotyledonous and dicotyledonous weeds. These two endothall formulations were restricted to lake and pond applications; however, in 2010, endothall received a label for use in flowing water for vascular plant and algae control (EPA 2010). The flowing water commercial products were Cascade® (dipotassium salt of endothall) and Teton® [mono(N,N-dimethylalkylamine) salt of endothall] and provided an alternative to acrolein (Magnacide™ H) for aquatic weed management in irrigation and drainage canals. The amine formulation is generally two to three times more active than the dipotassium formulation on algae and macrophytes, but it is also 200 to 400 times more toxic to nontarget aquatic organisms such as fish (Sprecher et al. 2002).

Endothall is considered a contact herbicide (Madsen 1997; Shaner 2014) and is in its own chemical class. Endothall's mode of action was unknown for over sixty years, but recently its mode of action was identified as serine/threonine protein phosphatase inhibitor (Tresch et al. 2011; Bajsa et al. 2012). Endothall is a broad-spectrum herbicide that is effective against monocotyledons and dicotyledons weeds (Madsen 1997; Westerdahl and Getsinger 1988). Under both aquatic and terrestrial conditions visual symptoms are similar to those of chilling injury with defoliation and brown, desiccated tissue, growth inhibition, and root swelling (Tresch et al. 2011; Shaner 2014).

Endothall has been widely used to control Eurasian watermilfoil (*Myriophyllum spicatum* L.) (EWM) and hydrilla [*Hydrilla verticillata* (L.f.) Royle] throughout the United States (Skogerboe and Getsinger 2001, 2002). These invasive plants can form extensive, undesirable surface canopies that can negatively affect water quality, native plant communities by limiting light penetration, significantly reducing dissolved oxygen, and increasing water temperature. These dense infestations can also impact recreational uses of a water body such as swimming, fishing, and boating (Netherland et al. 2000; Bowes et al. 1979).

Hydrilla is an aggressive submersed weed. It is one of the most difficult invasive aquatic weeds to manage in the United States. Two different hydrilla biotypes exist in the US, a triploid monoecious and a triploid dioecious biotype. The dioecious biotype is more commonly found in the southern US and it was the first biotype introduced, while the monoecious biotype is more commonly found from North Carolina northward (Dayan and Netherland 2005; True-Meadows et al. 2016). Hydrilla is very sensitive to the systemic herbicide, fluridone (Dayan and Netherland 2005), a phytoene desaturase inhibitor that cause hydrilla tuber numbers to decrease over time (Nawrocki et al. 2016).

The susceptible hydrilla phenotypes can be controlled by fluridone at rates as low as 4 μg L^{-1}, so fluridone was intensively used for decades. After years of repetitive treatments, fluridone-resistant dioecious hydrilla was reported in Florida requiring up to 30 μg L^{-1} of fluridone for complete hydrilla control (Michel et al. 2004). To date, only the female form of dioecious hydrilla has been found in the US and the realization that a plant that relies entirely on vegetative reproduction could evolve herbicide resistance was a driving force behind identifying and registering several new herbicide modes of action for aquatic plant management.

EWM is sometimes referred to as the hydrilla of the northern US. It infests a much larger area relative to hydrilla. EWM was introduced in the United States in the 1940s (Couch and Nelson 1985) and it is one of the most economically costly aquatic plants to manage (Pimentel 2009). After its introduction, this submersed aquatic macrophyte spread rapidly throughout the US and it is present in at least 49 states (USGS 2018). EWM is an evergreen perennial plant that produces viable seed, but its invasiveness is mostly due to autofragmentation (Smith and Barko 1990). It usually occurs soon after flowering and new fragments often fall to the bottom or float off to form new infestations (Vassios et al. 2011). One single node is enough to start a new plant and eventually a new population (Grace and Wetzel 1978).

Recently, commercial applicators in the upper mid-west identified EWM with reduced sensitivity to 2,4-D (Larue et al. 2013). Genetic analysis determined that these plants were hybrids between native northern watermilfoil (*M. sibiricum* Komarov) and EWM. In addition to reduced herbicide sensitivity, these hybrid watermilfoil biotypes are more invasive, with significantly higher growth rates compared to either parent (Moody and Les 2007).

Endothall is an alternative mode of action for EWM and hybrid milfoil control; however, from an operational perspective there have been some concerns about using a contact herbicide rather than one that has systemic activity to manage EWM. There is some evidence that endothall has systemic activity (Thomas and Seaman 1968), but there has never been a definitive determination about endothall’s behavior in EWM and hydrilla. Herbicide translocation to roots is important, especially for long-term control of perennial species such as EWM and hydrilla. Therefore, the objectives of this research were to determine endothall’s (1) maximum shoot absorption; (2) shoot to root translocation; and (3) desorption in EWM and two hydrilla biotypes.

MATERIALS AND METHODS

Absorption and Translocation

EWM shoot fragments were collected from the Leggett Canal, north of Boulder, CO (4013’ N, 10508’ W) in fall 2006, and cultured under greenhouse conditions for the last 12 years. In order to produce uniform plant material for the research, apical sections from the previously propagated plants were cut into 10 cm pieces and the distal end was planted in 16 cm x 12 cm x 6 cm (1152 cm^3) plastic pots filled with field soil and 1 cm of sand was placed on the top. Each pot was fertilized with 2 g of slow release fertilizer (Osmocote Classic 19-6-12, Everris NA, Inc., USA) and six apical meristems shoots were planted in each pot. Plants were grown in dechlorinated tap water in 1.2 m x 1 m x 0.9 m (1041 L) plastic tanks in the greenhouse until they produced roots. The photoperiod was 14:10 h light:dark, supplemental lightening was provided with 400-watt sodium halide light bulbs, and the greenhouse temperature was set at 24 C during the day and 18 C at night.

When shoots reached 15 cm in length, they were removed from their original pots and plants with the well-developed roots were selected for absorption and translocation experiments. Roots were rinsed with tap water to remove any soil residue, and replanted in 15 mL plastic test tubes (15 mL Conical Centrifuge Tubes, Thermo Fisher Scientific, USA). Test tubes were filled with unwashed silica sand. After transferring the plants into test tubes, a low melting point eicosane wax (Eicosane 99%, Fisher Scientific, USA) was used to seal the top of the tube to isolate the root system from water column. Plants were transferred to 4 L glass beakers (25 cm tall X 15 cm diameter) filled with 3.5 L of dechlorinated tap water and were allowed to equilibrate for 24 h prior to treatment with ^{14}C-endothall.

Monoecious and dioecious hydrilla plants were propagated from tubers collected from Shearon Harris Lake, North Carolina, and Orange Lake, Florida, respectively. Tubers were kept in tap water in the greenhouse for two weeks and germinated tuber of similar size were transferred to field soil to grow, and then to test tubes as previously described. Monoecious hydrilla plants were smaller, approximately 10 cm long, and had fewer roots than the other two species.

Six beakers were treated with formulated endothall (Cascade®, United Phosphorus, Inc.) combined with ^{14}C-endothall (11.24 MBq mg^{-1} specific activity). The treatment solution was prepared by adding 409 KBq of ^{14}C-endothall to 127 µL of formulated endothall. Each beaker was then treated with 43 µL of treatment solution, containing 66 KBq of ^{14}C-endothall and enough formulated endothall to achieve a final concentration of 3 mg L^{-1}. The amount of ^{14}C-endothall in each beaker was determined by taking a 5 ml aliquot to determine disintegration per minute (dpm)/mL of treatment solution by liquid scintillation spectroscopy (LSS) (Packard 2500R, PerkinElmer, USA).

The eighteen plants needed for each experiment were separated into three beakers and each beaker contained a different species/biotype. Round plastic test tube racks were used to hold plants and a stir bar was placed underneath each rack. Plants were maintained in the laboratory, at 22 C, with 12:12 h light:dark period, supplemented with two fluorescent grow lights. Beakers were stirred three times a day for 30 min each time. Plants were harvested at 6, 12, 24, 48, 96 and 192 h after treatment (HAT). Three replicates of each species were randomly harvested from a different beaker at each time point, triple rinsed in non-treated dechlorinated tap water, divided into aboveground and belowground parts, for determination of fresh weights. Plant parts were dried at 60 C for 48 h to determine dry biomass. Plant tissues were combusted in a biological oxidizer (OX500, R.J. Harvey Instrument Co., USA) for 2 min. The $^{14}CO_2$ was collected by a CO_2 trapping cocktail (OX161, R.J. Harvey Instrument Co., USA). The oxidizer efficiency was determined before oxidizing plant parts and it was always greater than 90%. After oxidation, radioactivity was quantified by LSS. The study was repeated twice.

Desorption

To determine endothall desorption three beakers filled with 1 L of dechlorinated tap water were treated as described before. The treatment solution was prepared by adding 350.7 KBq of ^{14}C-endothall in 17.8μL of formulated endothall (Cascade®, United Phosphorus, Inc.). Each beaker was then treated with 35.9μL of treatment solution, containing 116.9 KBq of ^{14}C-endothall and enough formulated endothall to achieve a final concentration of 3 mg L^{-1} in the treatment solution. To confirm the amount of ^{14}C-endothall present in the treatment solution, 5 ml of treated water were removed and analyzed by LSS. Each beaker contained 15, 10 cm apical meristem shoots of one species and a stir bar. During the experiment, plants were maintained in the laboratory, at 22 C, with 12:12 h light:dark period, supplemented with two fluorescent grow lights. Plants were allowed to absorb endothall for 24 h, they were then triple rinsed in non-treated water, and placed in falcon tubes containing 40 ml of dechlorinated tap water. The amount of endothall desorbing from treated shoots was determined by removing shoots from clean water after 0, 12, 24, 48, 96 h and oxidizing each shoot as previously described. Water samples were taken from each falcon tube, and radioactivity was determined using LSS. The study was repeated twice.

Statistical Analysis

Levene's test for homogeneity of variance was performed using R (Version 3.3.1, R Project) to determine if data from repeated experiments could be combined. Based on results of Levene's test for homogeneity of variance (α=0.05 level of significance), data from repeated experiments were combined for statistical analysis. Means and standard errors for each experiment were back calculated from dry weight, considering 90% of water content, using MS Excel (MS Office 2016). Data were plotted with the use of SigmaPlot (Version 14, SYSTAT, 2017) and nonlinear regression analyses were also conducted to fit shoot absorption data to asymptotic rise to max function shown below (Kniss et al 2011):

$$\mathrm{Y} = \frac{ax}{b+x}$$

In addition to nonlinear regression analyses, for absorption and translocation data, the percentage of total herbicide present in aboveground and belowground plant parts was calculated to determine translocation, and the plant concentration factor (PCF) was calculated to determine herbicide bioconcentration. The equation used to calculate PCF was presented in Vassios et al. (2017) as adapted from de Carvalho et al. (2007) and was defined as:

$$\mathrm{PCF} = \frac{Herbicide\ concentration\ in\ plant\ (KBq/g\ fresh\ biomass)}{Herbicide\ concentration\ in\ water (KBq/mL)}$$

RESULTS AND DISCUSSION

Endothall Absorption

Endothall absorption did not reach an A_{max}, or maximum asymptote, in monoecious hydrilla or dioecious hydrilla even though plants were exposed to the herbicide for a 192 h time course. Endothall absorption by dioecious hydrilla was linear, which was an interesting and unexpected finding (Figure 1). Kniss et al. 2011 described how the asymptotic rise to max function is the most biologically relevant function to describe herbicide absorption. Endothall absorption by EWM fit the more typical asymptotic rise function, reaching an A_{max} by 192 h (Figure 1).

The reasons for greater endothall accumulation in dioecious hydrilla and not reaching a maximum asymptote are unknown, but one possible explanation could be rapid endothall metabolism, allowing plants to absorb endothall by diffusion resulting from a continuous concentration gradient. It is also important to note that the study was conducted under laboratory conditions and in a static water system where the herbicide concentration was maintained for the duration of the study. Under field conditions herbicide concentrations would decrease during this time frame because the herbicide would be diluted or degraded. Endothall half-life under field condition range is 1 to 7 d (Langeland and Warner 1986).

Endothall's water solubility (100,000 mg L^{-1}) and log K_{ow} (-0.55) suggest that accumulation in hydrilla and EWM would not be significantly greater than the external water concentration. For example, imazamox (water solubility 4,413 mg L^{-1} and log K_{ow} 0.73) accumulation in EWM was essentially equivalent to the external herbicide concentration, plant concentration factor (PCF) ≅ 1 (Vassios et al. 2011). Dioecious hydrilla, monecious hydrilla, and

EWM accumulated endothall above the concentration in the treatment solution. After 192 h, the PCF for these three species were 11.00 ± 0.94, 6.59 ± 0.74, and 3.28 ± 0.43 PCF, respectively (mean ± SE) (Table 1).

Endothall has a log K_{ow} very similar to triclopyr, -0.55 and -0.45, respectively, and endothall and triclopyr had very similar PCF values in the two hydrilla biotypes, but in EWM triclopyr's accumulation was 10 times greater than endothall (Vassios et al. 2017). Penoxsulam, another hydrophilic herbicide (log K_{ow} -0.35), had similar accumulation in EWM to endothall (Vassios et al. 2017). PCF values for fluridone and endothall were similar in hydrilla; however, in EWM, fluridone had a higher PCF compared to endothall, 19.97 compared to 3.28, respectively. Based on log K_{ow} values fluridone should accumulate more than endothall, so the fact that endothall accumulates in EWM and the two hydrilla biotypes significantly above the concentration in the treatment solution is notable. De Carvalho et al (2017) found that log K_{ow} values are not reliable predictors of herbicide accumulation in aquatic plants when those values are <2.

Increased herbicide accumulation does not always equate to better control; however, previous studies have demonstrated that dioecious hydrilla is more sensitive to endothall than monoecious hydrilla (Poovey and Getsinger 2010). Our results support previous studies showing higher total absorption and absorption rates in dioecious hydrilla leads to better control and greater biomass reduction.

Endothall Translocation

Shoot-to-root translocation in EWM was 8.00 ± 1.26% of total absorbed radioactivity 192 HAT (Figure 2). Penoxsulam, fluridone, and triclopyr translocation to the belowground EWM tissue 192 HAT was 1.3, 2.0 and 2.6% of total absorbed herbicide, respectively, illustrating that endothall's translocation was 6.1, 4, and 3.1 times greater than these systemic herbicides, respectively (Vassios et al. 2017).

Endothall's translocation to the roots in monoecious and dioecious hydrilla was 17.83 ± 5.07% and 16.40 ± 2.30%, respectively (Table 1). Vassios et al. (2017) found 6.1, 9.0 and 12.5% of total absorbed penoxsulam, fluridone, and triclopyr, respectively, being translocated to dioecious hydrilla roots. Endothall's translocation to hydrilla roots was greater than for penoxsulam, fluridone, and triclopyr. When endothall was applied to a leaf of longleaf pondweed (*Potamogeton nodosus* Poir.), some herbicide moved into the stem and accumulated mostly in the youngest leaves, but did not move into mature leaves or roots (Thomas and Seaman 1968). In the same study, endothall applied to longleaf pondweed roots distributed to the whole plant after 3 d, demonstrating that the herbicide moved from roots to shoots. Endothall translocation could be species dependent.

Turgeon et al. (1972) found a significant amount of endothall being translocated from leaves to roots for two terrestrial species, Kentucky bluegrass (*Poa pratensis* L. 'Merion') and annual bluegrass (*Poa annua* L.). Our results provide additional support to endothall's systemic activity by demonstrating its translocation in the aquatic plants: EWM, monoecious hydrilla, and dioecious hydrilla.

Endothall Desorption

Herbicide absorption continues to increase over time in these controlled environment experiments because there is no herbicide dilution or degradation; however, it is important to note that herbicides can diffuse out of the plant when external herbicide concentrations decrease (Vassios et al 2011). When treated plants were transferred to non-treated water, endothall desorption was lower than expected for all three species. Based on endothall's water solubility

(100,000 mg L^{-1} at 20 C), we hypothesized that it would rapidly equilibrate with the water column when treated shoots were exposed to clean water. Endothall's desorption was calculated as a percentage of total endothall absorbed on a whole plant basis. Monoecious hydrilla, dioecious hydrilla, and EWM desorbed 16.99 ± 8.26 %, 18.05 ± 4.11 % and 28.92 ± 15.63 %, respectively, of the absorbed endothall 96 HAT (Figure 3). Although total desorption was low for all three species, the majority of desorption occurred within a relatively short time frame of 12 HAT for all species. Herbicide movement out of treated plant tissue has only been studied in a few aquatic species. Imazamox desorption was evaluated in EWM (Vassios et al, 2011). Imazamox reached equilibrium (50:50 ratio) with non-treated water faster than endothall. Imazamox is a systemic herbicide with a log K_{ow} of 0.73 and 4,400 mg L^{-1} water solubility. Because of these chemical properties, imazamox would be expected to desorb quickly. After 12h of exposure to clean water, 46% of absorbed imazamox moved out of EWM shoots (Vassios et al. 2011). Imazamox desorption is driven mainly by a concentration gradient where the plant and the water column establish equilibrium. These data provide additional evidence to our hypothesis that endothall is being rapidly metabolized by the plant into a more lipophilic, conjugated, or insoluble metabolite, particularly by both hydrilla biotypes, considering that endothall desorption in hydrilla was ~10% lower than EWM.

In conclusion, endothall's total accumulation in plant tissue was not fast, but significantly higher than the concentration in the treatment solution for all three species. Endothall was translocated to the roots of Eurasian watermilfoil, monoecious and dioecious hydrilla to a greater extent than three systemic herbicides, fluridone, penoxsulam, and triclopyr, based on percentage of herbicide absorbed. Endothall desorption was less than 30% in all three species, and much lower than predicted based on its water solubility. These data provide strong evidence that endothall behaves as a systemic herbicide in these aquatic species.

Table 2.1: Plant concentration factor (PCF), endothall distribution in plant parts, and parameters for distribution of ^{14}C. Values represent the mean, and error terms represent the standard error of the mean (n = 6).

Species	Plant Part	PCF_{192}	$Distribution_{192}$ (%)	a ± SE	b ± SE
Eurasian watermilfoil	Aboveground	3.28 ± 0.43	92 ± 14.2	202.3 ± 28.05	231.6 ± 50.47
	Belowground		8 ± 1.3	8.526 ± 0.767	20.7 ± 6.231
Monoecious hydrilla	Aboveground	6.59 ± 0.74	82 ± 14.8	146.7 ± 18.08	138.3 ± 31.44
	Belowground		18 ± 5.1	52.29 ± 28.86	360.3 ± 258.3
Dioecious hydrilla	Aboveground	11.00 ± 0.94	84 ± 10.3	436.8 ± 180.8	804.7 ± 396.9
	Belowground		16 ± 2.3	226.8 ± 121.9	2429 ± 1676

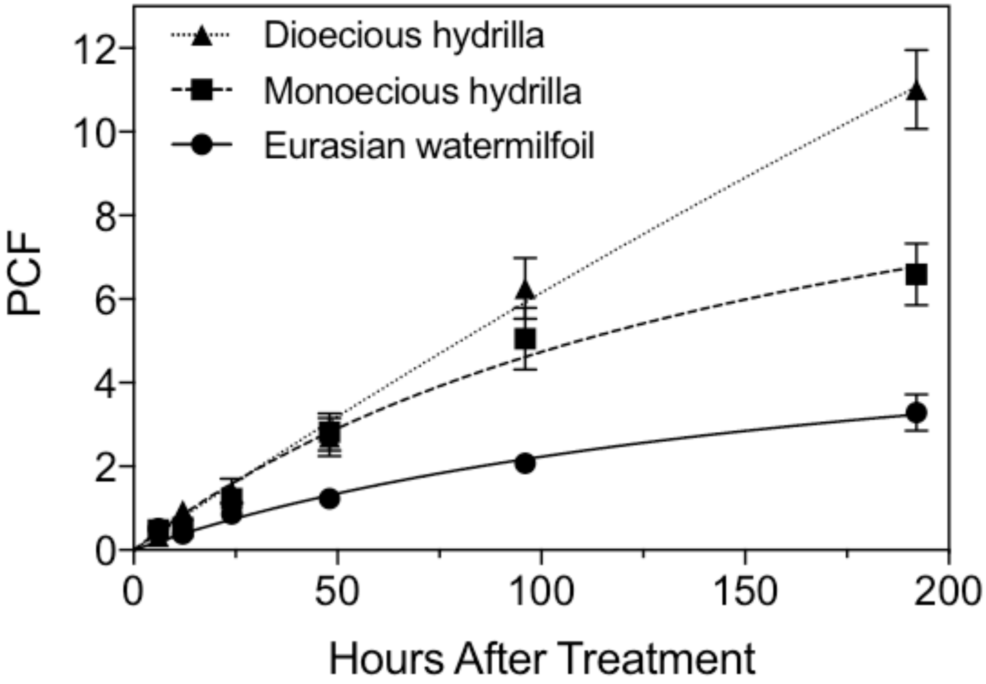

Figure 2.1: Endothall concentration in plants over 192 h, expressed as plant concentration factor, divided into 3 species: (1) dioecious hydrilla (y = 85.73*x / 1294+x, $r^2 = 0.9791$); (2) monoecious hydrilla (y = 12.73*x / 168.8+x, $r^2 = 0.9524$); and (3) Eurasian watermilfoil (y = 6.372*x / 185.5+x; $r^2 = 0.9484$). Data presented are means, and error bars are the standard error of the mean (n = 6).

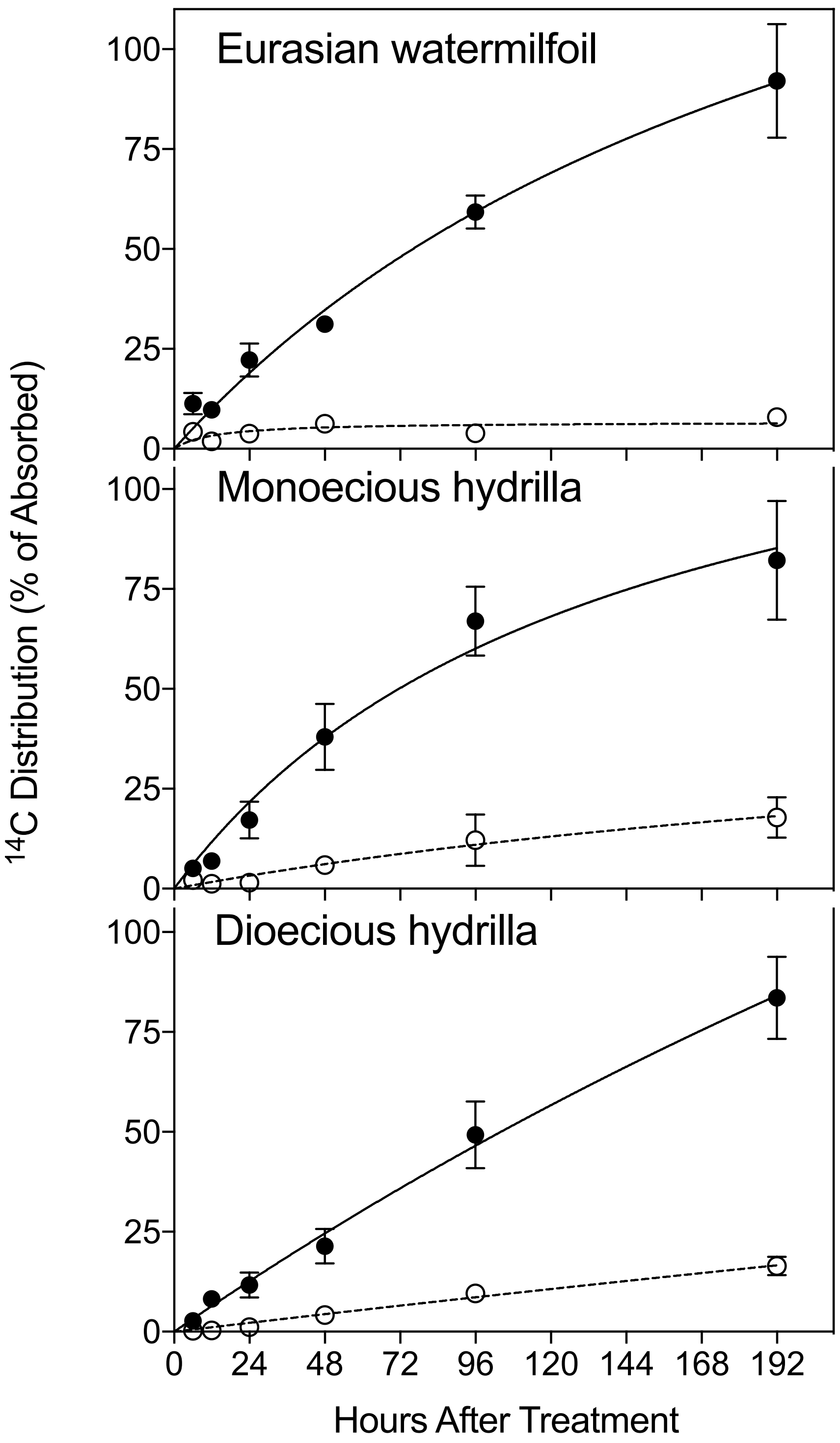

Figure 2.2: ^{14}C distribution in plants over 192 h following exposure to ^{14}C-endothall, expressed as percentage of total herbicide absorbed. Closed circles are the percentage of herbicide in the shoots; open circles are the percentage of herbicide in the roots. Data presented are means, and error bars are the standard error of the mean (n = 6).

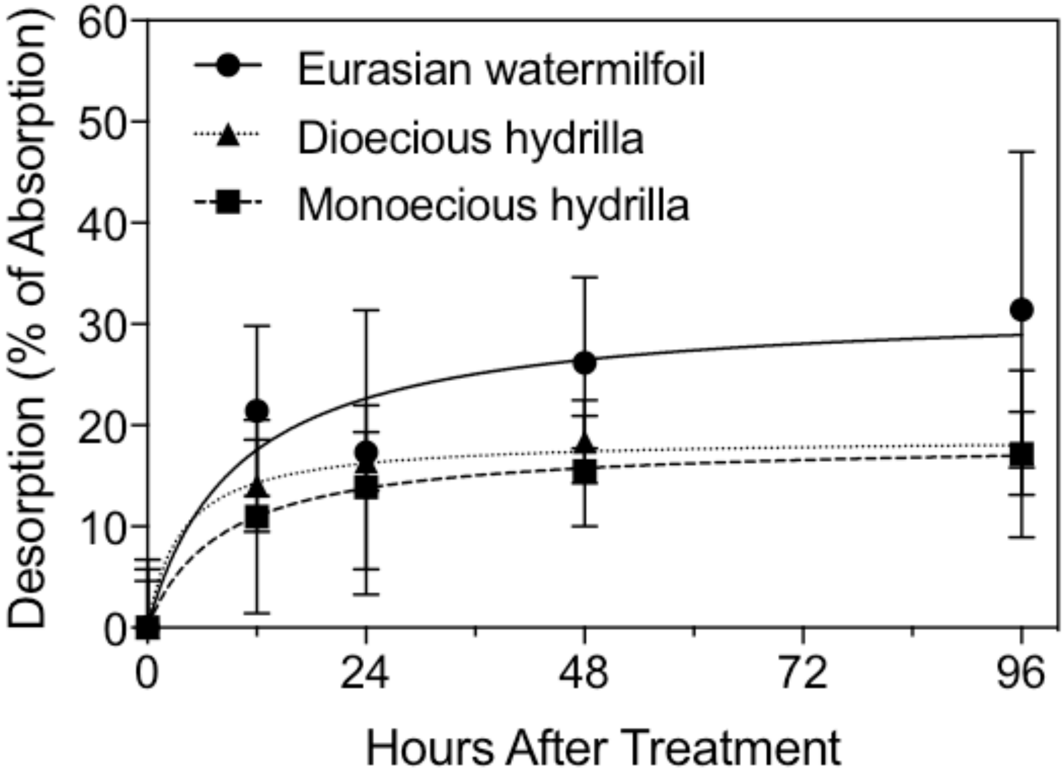

Figure 2.3: Desorption of ^{14}C-endothall over 96 h, expressed as a percentage of total absorbed ^{14}C following a 24 h treatment to 3 mg L^{-1} endothall, divided into 3 species: (1) Eurasian watermilfoil ($y = 31.87*x / 9.786+x$); (2) dioecious hydrilla ($y = 18.76*x / 3.741+x$); and (3) monoecious hydrilla ($y = 18.44*x / 8.138+x$). Data presented are means, and error bars are the standard error of the mean ($n = 6$).

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Chapter 3: Endothall Absorption and Translocation in Curlyleaf Pondweed and Sago Pondweed

INTRODUCTION

Invasive aquatic plants negatively impact natural processes and human activity (Netherland et al. 2000; Bowes et al. 1979). To mitigate these negative impacts, a range of mechanical, cultural, physical, and biological control methods are implemented for submersed aquatic weed management. One of the most commonly used and often the most cost-effective methods for selective management of invasive aquatic plants is the use of aquatic herbicides. There are approximately 225 herbicides according to the WSSA Herbicide Handbook (Shaner 2014); however, only 14 herbicides are available for aquatic weed control. Endothall is one of 15 herbicides that are labeled for aquatic applications. It was initially registered for terrestrial uses in the 1950s and was not labeled for aquatic use in lakes and ponds until 1960 (Hiltibran 1962). In 2010, endothall's label was expanded to include uses in irrigation and drainage canals (EPA 2010). Endothall is primarily used to control submersed weeds.

Despite being labeled as a terrestrial herbicide since the 1950s and an aquatic herbicide since the 1960s, endothall's mode of action was unknown for over sixty years. Endothall is considered a contact herbicide in its own chemical class (Madsen 1997; Shaner 2014). Endothall is a serine/threonine protein phosphatase inhibitor (Tresch et al. 2011; Bajsa et al. 2012) and is a broad-spectrum herbicide effective against monocotyledons and dicotyledons (Westerdahl and Getsinger 1988; Madsen 1997). Some of the key species controlled by endothall are milfoil

(*Myriophyllum* spp.), pondweed (*Potamogeton* spp.), hydrilla [*Hydrilla verticillata* (L.f.) Royle], coontail (*Ceratophyllum* spp.) and naiad (*Najas* spp.).

Most invasive aquatic plants, like the species listed above, can form extensive, undesirable canopies that can negatively affect water quality, native plant communities by limiting light penetration, significantly reducing dissolved oxygen, increasing water temperature, and they can also impact recreational activities such as swimming, fishing and boating (Bowes et al. 1979; Netherland et al. 2000). Boating, fishing, and general tourism are extremely important to the economies of states like Vermont, New Hampshire, Wisconsin, Washington, Idaho, and Florida. Boating alone is a billion dollar a year business, supporting nearly a million jobs, 35,000 businesses, and annual spending of $83 billion (NMMA, 2013). Large curlyleaf pondweed (*Potamogeton crispus* L.) (CLP) infestations can impede water flow by more than 90% in rivers, damage water conveyance equipment and ultimately impede navigation (Bolduan et al. 1994).

CLP is a submersed, aquatic macrophyte, native to Europe, Asia, Africa and Australia. The first report of CLP in the US was from Delaware in 1859 (Stuckey 1979). It is an herbaceous perennial monocotyledonous plant that senesces as plants go dormant in the summer. Although it is a perennial species, it has characteristics of an annual species as it senesces completely in early summer and only the turions and seeds “over-summers” (Netherland et al. 2000). This species was named after its wavy leaf structure, making it easy to identify.

CLP can thrive in a wide range of growing conditions, from very warm summer temperatures to ice-covered water with very low light intensities (Gettys et al. 2014). CLP is considered a cool water plant with a unique life cycle for submersed aquatic plants. It reproduces primarily by producing turions (hardened modified reproductive buds that form from apical buds and leaf axils) and rhizomes, but it can also produce viable seeds (Barr and DiTomaso 2014).

Plants achieve their maximum density in late spring, which is when they flower and produce turions, before plants senesce in early summer. Turions are dormant during the summer and sprout in the fall with shorter daylength and cooler water temperatures (Netherland et al. 2000).

CLP is susceptible to endothall. Treating CLP with endothall in the spring when water temperatures are lower (16 C) provided better control than treating when water temperatures were warmer (23 C), 90% control compared to 60% respectively (Poovey et al. 2002). In the same study no live plants were observed 6 weeks after treatment, but plant regrowth was observed by 12 weeks after treatment. Netherland et al. (1991) reported similar reductions in CLP biomass with endothall (>85%) and no regrowth for at least 4 weeks.

Sago pondweed (*Potamogeton pectinatus* L.) (SPW) is in the same plant family as CLP, Potamogetonaceae. It is a submersed macrophyte that is native to the US, but can be found worldwide in temperate regions. In these climates, SPW is one of the first species to grow in the spring, giving it an advantage when compared to native species that start actively growing a few months later (Kantrud 1990). Although it can cause localized problems in lakes, SPW is a major problem in flowing water, such as irrigation and drainage channels. Once established, the plant forms dense, monotypic stands that can choke irrigation canals and significantly reduce water flow. SPW spreads primarily through extensive rhizomes, but it persists by the production of subterranean tubers. One single plant can produce tens of thousands of tubers in one growing season, making it a very prolific colonizer (Yeo 1965).

SPW and CLP are highly susceptible to endothall (Westerdahl and Getsinger 1988; Slade et al. 2008), which would appear to go against the paradigm that systemic herbicides are needed to control perennial aquatic plants. Our goal was to provide additional evidence that endothall is actually a systemic herbicide as suggested by Thomas and Seaman (1968). Endothall’s behavior was evaluated in CLP and SPW with the objective of (1) determining endothall’s maximum absorption and absorption rate and (2) quantifying endothall translocation.

MATERIALS AND METHODS

Plant Material

CLP turions were collected in fall 2016 from Spring Gulch Pond, in southwest Denver, Colorado (39^{o} 32’49.52” N 105^{o} 02’36.07” W). In order to produce uniform plant material, turions of similar size were planted in 16 cm x 12 cm x 6 cm (1152 cm^3) plastic pots filled with field soil and 1 cm of sand on top. Each pot was fertilized with 2 g of slow release fertilizer (Osmocote Classic 19-6-12, Everris NA, Inc., USA) and six turions were planted in each pot. Plants were grown in dechlorinated tap water in 1.2 m x 1 m x 0.9 m (1041 L) plastic tanks for approximately four weeks. The photoperiod was 14:10 h light:dark, supplemental lightening was provided with 400-watt sodium halide light bulbs, and the greenhouse temperature was set at 24 C during the day and 18 C at night.

When CLP shoots reached 15 cm in length, plants with the most developed roots were selected, removed from their original pots, roots and turions were rinsed with tap water to remove any soil residue, and replanted in 50 mL plastic test tubes (50 mL Conical Centrifuge Tubes, Thermo Fisher Scientific, USA) filled with unwashed silica sand. Prior to transferring the plants, the test tubes were cut down to 35 mL, to ensure that the shoots were fully exposed to treated water. After transferring the plants into test tubes, a low melting point eicosane wax (Eicosane 99%, Fisher Scientific, USA) was used to seal the top of each tube to isolate the root system from water column. Plants in test tubes were transferred to clear 4 L glass beakers (25 cm tall X 15 cm diam.) filled with 3.5 L of dechlorinated tap water and were allowed to equilibrate for 24 h prior to treatment with ^{14}C-endothall.

SPW plants were propagated from tubers purchased from Kester's Nursery (P.O. Box 516, Omro, WI, 54963). Tubers of similar size were planted into field soil to grow and when they reached 15 cm in length, they were transferred to test tubes as previously described.

Herbicide Exposure

Four liter glass beakers were filled with 3.5 liters of dechlorinated tap water and treated with formulated endothall (Cascade®, United Phosphorus, Inc.) combined with ^{14}C-endothall (11.24 MBq mg^{-1} specific activity), there were 3 tanks per species for a total of 6 treatment tanks. The treatment solution was prepared by adding 409 KBq of ^{14}C-endothall to 127µL of formulated endothall. Each beaker was then treated with 43µL of treatment solution, containing 20µL formulated endothall and 66 KBq of ^{14}C-endothall to achieve a final concentration of 3 mg L^{-1}. To confirm the amount of ^{14}C-endothall present in the treated tanks, 5 mL were collected from each tank, transferred to 20 mL scintillation vial containing 10 mL of scintillation solution (Ecoscint XR, National Diagnostics, USA), vortexed, and radioactivity was quantified using a liquid scintillation spectroscopy (Packard 2500R, PerkinElmer, USA) (LSS). Three beakers contained six CLP plants each, and three beakers contained six SPW plants, all plants were held by a plastic round test tube rack at the bottom of each beaker, and a stir bar was placed underneath the racks. During the experiment, plants were maintained in the laboratory, at 22 C, with 12:12 h light:dark period, supplemented with two fluorescent grow lights, and beakers were stirred two times a day for 30 min. Following treatment, plants were harvested at 6, 12, 24, 48, 96 and 192 h after treatment (HAT). Three replicates per species were randomly harvested from a different tank at each time point, triple rinsed in non-treated dechlorinated tap water, divided into aboveground and belowground parts, and fresh weight was recorded. Plant parts were dried at 60 C for 48 h to achieve constant moisture, dry biomass was recorded for each plant part, plant tissues were combusted in a sample oxidizer (OX500, R.J. Harvey Instrument Co., USA) for 2 min, and absorbed ^{14}C was collected by a ^{14}C trapping cocktail (OX161, R.J. Harvey Instrument Co., USA). Oxidizer efficiency was tested before oxidizing plant parts and it was always greater than 90%. The study was repeated.

Statistical analysis

For each study, Levene's test for homogeneity of variance was used to determine if data from repeated studies could be combined for statistical analysis (α=0.05 level of significance). Based on results of Levene's test for homogeneity of variance data from repeated experiments were combined for statistical analysis. Means and standard errors for each experiment were calculated using MS Excel (MS Office 2016). Data were plotted with the use of SigmaPlot (Version 14, SYSTAT, 2017) and nonlinear regression analyses were also conducted to fit the hyperbolic function shown below:

$$Y = \frac{ax}{b+x}$$

Based on the predicted values from the asymptotic, one additional value was calculated (t_{90}), the predicted time it would take to reach 90% of that absorption.

In addition to nonlinear regression analyses, the percentage of total herbicide present in aboveground and belowground portions plant parts was calculated to determine translocation, and the plant concentration factor (PCF) was calculated to determine herbicide bioconcentration. The equation used to calculate PCF was presented in Vassios et al. (2017) as adapted from de Carvalho et al. (2007) and was defined as:

$$\text{PCF} = \frac{Herbicide\ concentration\ in\ plant\ (KBq/g\ fresh\ biomass)}{Herbicide\ concentration\ in\ water(KBq/mL)}$$

RESULTS AND DISCUSSION

Endothall Absorption

Although studies were conducted over a reasonably long time course of 192 h, endothall absorption did not reach a maximum asymptote in SPW, but it did in CLP, following the asymptotic rise to max function described by Kniss et al. (2011). The ratio of herbicide in the plant compared to the treatment solution (PCF) 192 HAT was 3.32 ± 0.72 for CLP, and 7.34 ± 0.57 PCF for SPW (Table 3.1). Endothall accumulation in EWM, monoecious hydrilla and dioecious hydrilla were 3.28 ± 0.43 PCF, 6.59 ± 0.74 PCF, and 11.00 ± 0.94 PCF, respectively (Chapter 2). The total endothall accumulation at 192 HAT was the same for CLP and EWM, 3.32 ± 0.72 PCF and 3.28 ± 0.43 PCF, respectively. Similarly, SPW and monoecious hydrilla had the same endothall accumulation at 192 HAT of 7.34 ± 0.57 PCF and 6.59 ± 0.74 PCF, respectively.

Similarly to endothall total accumulation in CLP and EWM, penoxsulam total accumulation in EWM was about 4 times more in the plant compared to the treatment solution (Vassios 2017). Likewise fluridone total accumulation in hydrilla was similar to endothall total accumulation at 192 HAT in SPW, 8.31 ± 0.66 PCF and 7.34 ± 0.57 PCF, respectively.

The rate of endothall accumulation in plant tissue, based on predicted t_{90} values, was faster for CLP, with 90% of total accumulation occurring at 98 HAT, while t_{90} occurred by 160 HAT in SPW (Table 3.1). Predicted t_{90} values for fluridone, triclopyr and penoxsulam in hydrilla were at 76, 113 and 145 HAT, respectively and 30, 110 and 73 HAT in EWM. (Vassios et al. 2017). So t_{90} values for endothall in CLP and SPW were in the same range as these other systemic herbicides.

SPW did not reach maximum endothall absorption by 192 HAT which could mean that the plant is rapidly metabolizing the herbicide. This would allow plants to continue absorbing endothall due to a continuous concentration gradient. It is important to note that the study was conducted under laboratory conditions and in a static water system where the herbicide concentration was maintained for the duration of the study. Under field conditions, especially considering that SPW is a major problem in flowing water systems, with high water exchange, herbicide concentrations would decrease during this time frame because the herbicide can be diluted or carried away.

Endothall Translocation to the Roots

Shoot-to-root translocation was limited in CLP and SPW reaching a maximum translocation at 192 HAT of 3.12 ± 1.08% and 1.16 ± 0.23% based on total absorbed radioactivity, respectively (Figure 3.2). Endothall translocation in EWM, dioecious hydrilla and monoecious hydrilla was 3.7, 7.7 and 8.3 times greater, respectively, than the average translocation in CLP and SPW (Chapter 2).

Endothall shoot-to-root translocation in CLP was greater than triclopyr, fluridone and penoxsulam translocation in EWM; however, the translocation of these three herbicides in EWM was greater than endothall translocation in SPW at 192 HAT (Vassios 2017). These results illustrate that the three systemic herbicides previously studied also had limited translocation to plant roots.

When endothall was applied to a longleaf pondweed (*Potamogeton nodosus* Poir.) leaf, some herbicide moved into the stem, accumulating mostly in the youngest leaves, indicating symplastic transport (Thomas and Seaman 1968). Limited endothall translocation by three pondweed species suggests that translocation could be species specific.

In conclusion, endothall accumulation in SPW and CLP tissue required between 98 and 160 HAT to reach t_{90}; however, significantly higher endothall concentrations were found in the plant compared to the treatment solution. Based on endothall's high water solubility and negative log K_{ow} PCF value was expected to be around 1 or equilibrium. Although endothall translocation to the roots of these two species was limited, it was very similar to the translocation of three systemic herbicides, fluridone, penoxsulam, and triclopyr, based on percentage of herbicide absorbed (Vassios et al. 2017). These data provide additional evidence that endothall is systemic and should be reclassified.

Table 3.1. Plant concentration factor (PCF), parameters, and calculated values based on hyperbolic regression analyses. Values represent the mean, and error terms represent the standard error of the mean (n = 6).

		Parameters and Estimates Based on Hyperbolic Regression Analyses		
Species	**PCF_{192}**	**t_{90} (h)**	**a ± SE**	**b ± SE**
Curlyleaf pondweed	3.32 ± 0.72	98	3.553 ± 0.2048	25.09 ± 4.567
Sago pondweed	7.34 ± 0.57	160	16.56 ± 2.517	251.6 ± 58.68

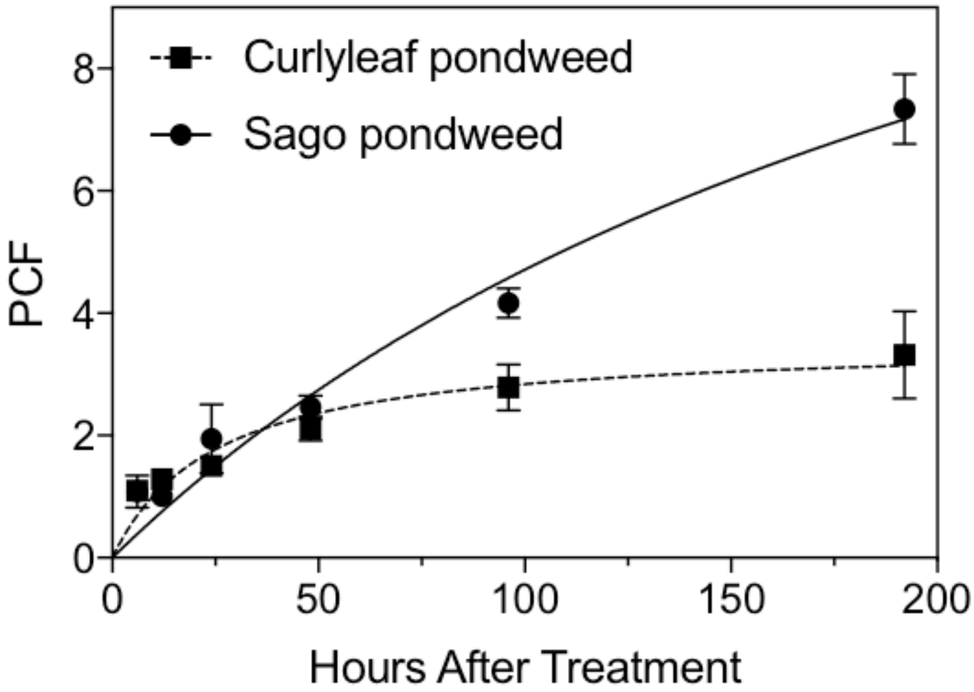

Figure 3.1: Endothall concentration in plants over 192 h, expressed as plant concentration factor (PCF): (1) curlyleaf pondweed (y = 3.553*x / 25.09+x, $r^2 = 0.7952$); and (2) sago pondweed (y = 16.56*x / 251.6+x, $r^2 = 0.9426$). Data presented are means and the standard error of the mean (n = 6).

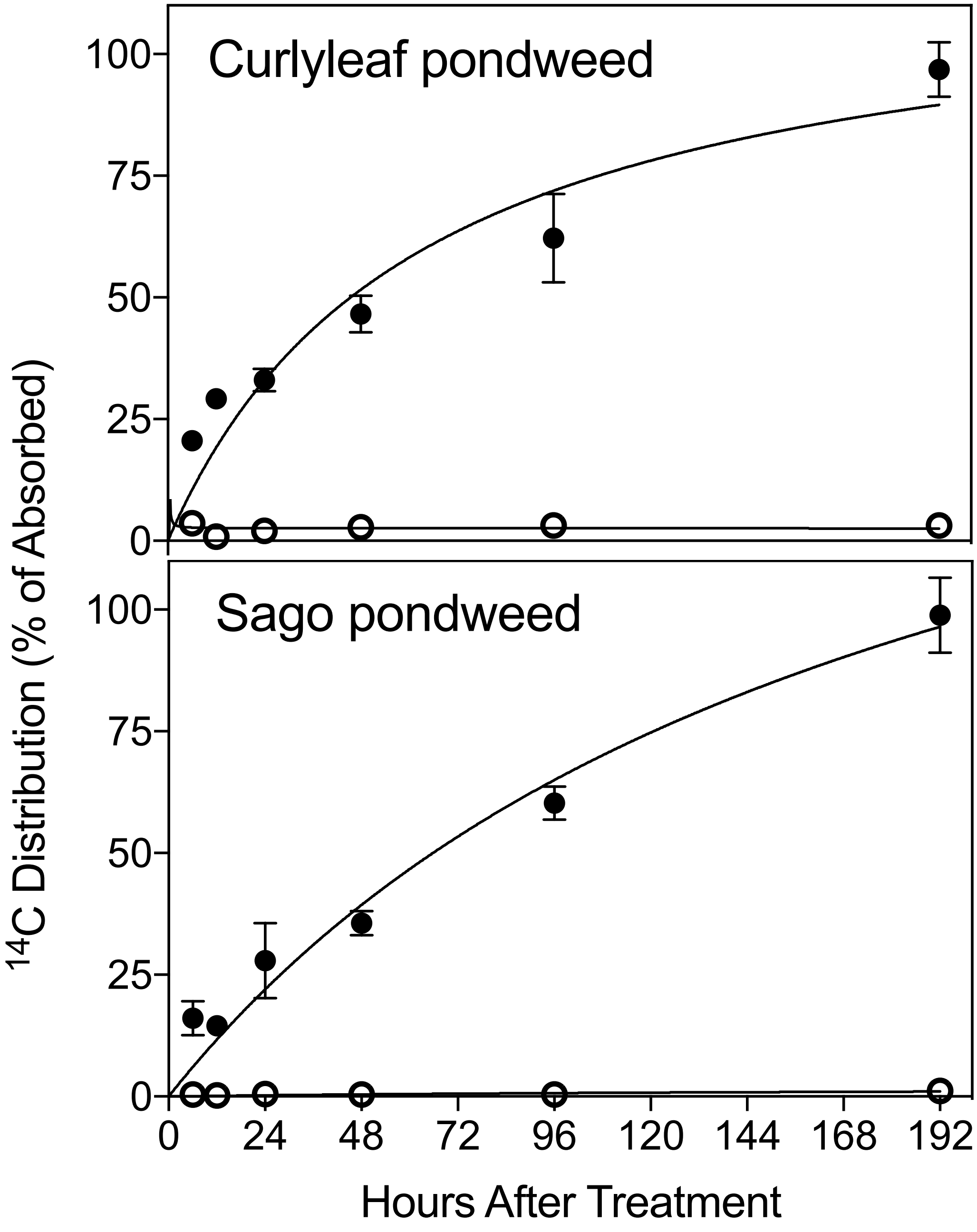

Figure 3.2: ^{14}C distribution in plants over 192 h following exposure to ^{14}C-endothall, expressed as percentage of total herbicide absorbed. Closed circles are the percentage of herbicide in the shoots; open circles are the percentage of herbicide in the roots. Data presented are means, and error bars are the standard error of the mean (n = 6).

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