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Evaluation of Metsulfuron-methyl for Giant Salvinia (Salvinia molesta) Control and Non-Target Species Sensitivity

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EVALUATION OF METSULFURON-METHYL FOR GIANT SALVINIA (*SALVINIA MOLESTA*) CONTROL AND NON-TARGET PLANT SPECIES SENSITIVITY

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Abstract

The invasive aquatic fern giant salvinia (*Salvinia molesta* D.S. Mitchell) has been invading waterbodies across Louisiana and Texas since 1998. Recently, the non-aquatic herbicide metsulfuron-methyl was found to be highly efficacious against giant salvinia, but limited information is available on application techniques, use rates and concentrations, as well as its impact on non-target species. Therefore, a series of mesocosm experiments were conducted to further evaluate foliar and subsurface applications of metsulfuron against giant salvinia, as well as foliar applications of metsulfuron in combination with aquatic herbicides commonly used for giant salvinia control. When applied to the foliage of giant salvinia, metsulfuron provided ≥ 98% giant salvinia control by 8 weeks after treatment (WAT) at 10.5 to 168.2 g active ingredient (a.i.) ha^{-1}. Metsulfuron was also compatible and highly efficacious in tank mix combinations with glyphosate, flumioxazin, diquat, and carfentrazone. All tank mixes increased speed of injury and plant death, but did not increase level of control. Subsurface applications of metsulfuron were also highly efficacious, and provided ≥ 98% control by 10 WAT at concentrations of 10 to 80 µg a.i. L^{-1}. The calculated LD_{90} and EC_{90} (lethal dose and effective concentration to control 90% of the test population) values were 3.83 g a.i. ha^{-1} and 1.87 µg a.i. L^{-1} for foliar and subsurface applications, respectively. Outdoor mesocosm trials were also conducted to evaluate the sensitivity of non-target aquatic and terrestrial plants to metsulfuron as a foliar treatment, or in irrigation water. Foliar applications of metsulfuron at ≥ 10.5 g a.i. ha^{-1} resulted in ≥ 98% reduction in plant biomass of giant blue iris (*Iris giganticaerulea* Small.) and broadleaf arrowhead (*Sagittaria latifolia* Willd.), and ≥ 84% reduction of yellow water lily (*Nymphaea mexicana* Zucc.). Broadleaf cattail (*Typha latifolia* L.) was only impacted by rates ≥ 42.1 g a.i. ha^{-1}. The non-target terrestrial plants vinca

[*Catharanthus roseus* (L.) G. Don.], soybean [*Glycine max* (L.) Merr.], and cherry tomato (*Solanum lycopersicum* L.) were not impacted by metsulfuron treated irrigation water at concentrations ranging from 1 to 40 μg a.i. L^{-1}, while iris was not affected until herbicide concentrations reached 40 μg a.i. L^{-1}.

Chapter 1. Introduction

1.1. History, Biology, and Ecology of Giant Salvinia

Giant salvinia (*Salvinia molesta* D.S. Mitchell) is a free-floating, aquatic fern native to Brazil that has become a nuisance in lakes, rivers, and reservoirs (Jacono 1999). Over the past century, giant salvinia has spread to tropical and subtropical regions around the world (Oliver 1993, Jacono and Pitman 2001). Due to its rapid growth rate, it negatively affects regions of Africa, Sri Lanka, New Guinea, the Philippines, and Australia (Oliver 1993, Chilton et al. 2002). The first documentation of established giant salvinia in the U.S. occurred in 1995 in South Carolina (Johnson 1995). This initial infestation was successfully eradicated from this site using aquatic herbicides (Nelson 2014); however, giant salvinia has since been reintroduced and reported in thirteen states including Alabama, Arizona, Arkansas, California, Florida, Georgia, Hawaii, Louisiana, Mississippi, North Carolina, South Carolina, Texas, and Virginia (Thayer et al. 2018). The introduction of the plant in the U.S. is likely due to the nursery trade and aquarium plant industry (Nelson 2014). Giant salvinia is listed as a Federal Noxious Weed by the U.S. Department of Agriculture, which prohibits importation to the U.S. and transportation across state lines (McFarland et al. 2004). A species must be listed as noxious by individual states to prohibit cultivation and sale of the species within that state, but the spread of giant salvinia is likely to continue across the U.S. since it is not listed as a noxious species in every state (Nelson 2014).

Giant Salvinia was first documented in Louisiana in the Toledo Bend Reservoir on the Texas-Louisiana border in late 1998 (Horst and Mapes 2000). An initial infestation in Louisiana estimated to be less than 162 hectares in 1999 has since grown to an estimated 18,340 hectares in 2013 (Mudge et al. 2014). In 2017, the Louisiana Department of Wildlife and Fisheries (LDWF) managed over 8,000 hectares of giant salvinia in Louisiana (Sartain 2018). The rapid spread of giant salvinia to new water bodies can be partially attributed to plant fragments transported by boats and trailers (Chilton et al. 2002). Plant fragments adhere to boats and trailers and then deposit to neighboring aquatic systems if boaters fail to properly clean their equipment. Giant salvinia can also spread into new areas by water current, flooding, and movement by wildlife (Horst and Mapes 2000).

Giant salvinia belongs to the Salviniaceae family, which consists of free-floating aquatic ferns coated with velvety hairs (trichomes) on the frond surfaces (McFarland et al. 2004). Giant salvinia forms free-floating colonies of potentially independent modules held together by a horizontal stem (rhizome) that floats just below the water surface (McFarland et al. 2004). A pair of floating fronds are produced at each node along the rhizome that are bright green, oval shaped, possess a central midrib, and are covered with rows of white, bristly trichomes (Nelson 2014). The trichomes are topped with four branches that from a structure resembling an "eggbeater" (McFarland et al. 2004). These hairs give the fronds a velvety appearance and serve as air traps to repel water and aid in flotation (Harper 1986). Nutrients are absorbed from the water column by delicate, finely dissected submersed fronds that resemble roots (Nelson 2014).

Water pH levels have a significant impact on the growth of giant salvinia. Previous research by Owens et al. (2005) demonstrated over a 2-fold increase in giant salvinia biomass at pH < 7.5 when compared to an environment with pH ranging from 8.5 to 10.0. Nutrients such as phosphorus, manganese, and iron can become bound in sediments at pH > 7.5, thus unavailable for floating plant uptake (Wetzel 1983). Since these nutrients are important for photosynthesis, chlorophyll synthesis, enzymatic activity, etc., availability is essential for healthy plant growth (Raven et al. 1981). When pH and dissolved oxygen concentrations decline, these sediment-bound nutrients may be released into the water column (Riemer 1984, Wetzel 1983). In these environments, optimum giant salvinia growth occurs at pH 6.0 to 7.5, in nutrient rich conditions with water temperatures ranging from 20 and 30° C (Cary and Weerts 1984).

While giant salvinia is able to produce sporocarps, the plant is characterized as pentaploidal, or possessing five homologous sets of chromosomes, and is believed to be genetically unable to produce fertile spores (i.e., asexual reproduction) (Loyal and Grewal 1966). Reproduction is accomplished by a parent plant breaking at the rhizome and producing two or more daughter plants (Room 1983). Fragmentation can occur by human activity, such as mechanical harvesting or boating, or by friction between plants transported by winds and water (Harley and Mitchell 1981, Room 1983, 1990).

Giant Salvinia exhibits growth in three distinct stages: primary, secondary, and tertiary (McFarland et al. 2004). The primary stage occurs during the initial invading phase of the infestation when plants are widely spaced and exhibit small, delicate leaves from 10 to 15 mm wide that lie flat on the surface of the water (Harley and Mitchell 1981). Plants may also exhibit this growth stage when recovering from damage (Harley and Mitchell 1981). Plants in the secondary stage of growth have grown in open water for some time and exhibit longer and larger stem internodes than primary stage plants, slightly cupped leaves (20 to 50 mm in diameter) that do not overlap, and the entire lower leaf surface is in contact with the water (McFarland et al. 2004). During the tertiary or mat forming stage, plants are under crowded conditions and exhibit large heart-shaped or oblong fronds up to 60 mm in diameter (Nelson 2014). As the plants continue to grow and become more crowded, the leaves are pushed upward and may form mats up to 1 m thick (McFarland et al. 2004).

Giant salvinia exhibits a very rapid growth rate that contributes to the invasive nature of the plant. Giant salvinia is capable of doubling its biomass in as little as 36 hours under optimal growing conditions (Johnson et al. 2010) with a single plant capable of covering 103.6 km^2 in as little as 3 months (Creagh 1991, 1992). Plants spread laterally within lakes through rhizome and lateral bud growth, while long distance dispersal is typically a result of fragmentation (Nelson 2014). With such a rapid growth rate, giant salvinia has the capability to outcompete desirable native vegetation for resources such as nutrients, light, and surface area (Mitchell and Tur 1975) and can quickly form a monoculture, inhibiting the growth of native aquatic plants that provide food and habitat for animals and waterfowl (Mitchell 1978). Once dense mats are formed, sunlight is shaded out, resulting in inhibited growth or death of the submerged vegetation. As the submerged plants and older giant salvinia die and decay, dissolved oxygen levels in the water are depleted, which forces fish and other aquatic animals to leave the area (Johnson et al. 2010). As a result, degradation of habitat for other aquatic plants, fish, invertebrates and wildlife occurs (Barrett 1989, Madsen 2014). Additionally, dense plant stands form a physical barrier on the water surface (McFarland et al. 2014) that can hinder activities such as boating, swimming, and fishing, while also impairing flood control, limiting irrigation, clogging water intakes, and decreasing waterfront property values (Nelson 2014). Other implications of giant salvinia infestations include a potentially devastating impact on rice production, crawfish and catfish farming operations, and waterfowl habitat (Horst and Mapes 2000). Public health problems are also a concern since giant salvinia serves as an important host plant and breeding habitat for mosquitoes (Room et al. 1989).

1.2. Giant Salvinia Management Methods

The key to managing giant salvinia is to recognize the problem early when the infestations are small and easier to contain (Nelson 2014). Once the infestation becomes well established, management options become much more difficult and expensive. Methods of controlling giant salvinia can be characterized as physical, biological, or chemical (Madsen and Wersal 2009). Selecting the best management strategy may depend on size of infestation, type of water body, accessibility to the infestation, and other variables.

Physical control options may include removing the target species by hand, employing the use of mechanical harvesters, using barriers such as floating booms or nets, destroying the plant by cutting, chopping or shredding targeted plants, or modifying the environment through methods such as lake drawdown (Chilton et al. 2002). Manual removal can be successful in the primary stage when plants are small and uncrowded (Thomas and Room 1986, Miller and Wilson 1989); however, once established, removal by hand is not a practical option due to the rapid growth rate and large amount of plant biomass (McFarland et al. 2004). Mechanical harvesters are typically very expensive due to the high degree of specialization required to collect and remove large quantities of biomass in an aquatic environment (Thomas and Room 1986, Chilton et al. 2002). The harvesters must be capable of removing biomass at rates exceeding regrowth in order to be effective (Storrs and Julien 1996). Considering the rapid growth rate of giant salvinia, this is virtually an impossible task to accomplish. Floating booms and nets have been used to prevent giant salvinia from clogging water intakes, boat launches, and swimming areas (Chilton et al. 2002). The drawback to this method of control is that booms and nets require constant attention and are subject to breakage under the pressure of large windblown mats (Oliver 1993). Since giant salvinia reproduces by fragmentation, the use of cutters, shredders, or choppers can actually spread the infestation by generating a large number of fragments (Madsen 2000, Chilton et al. 2002). Habitat alteration by water-level drawdown can be a relatively inexpensive method for controlling aquatic weeds (Chilton et al. 2002) since this method destroys the plant by drying or exposure to lethal freezing temperatures (Cooke et al. 1986). Surveys conducted by LDWF have demonstrated that a lake drawdown successfully decreased plant coverage from 1,800 hectares in July of 2008 to 340 hectares in February 2009 in Lake Bistineau, Louisiana (Louisiana Department of Wildlife and Fisheries 2016). While this method of control can be useful in some situations, it is limited to systems that have sufficient control structures present (Bellaud 2014), and is controversial since it limits activities such as boating, fishing, and hunting (Chilton et al. 2002). In addition, giant salvinia can survive short drawdown periods and can persist on moist soils (Nelson 2014); therefore, a relatively long drawdown period with limited rainfall would be necessary.

The salvinia weevil (*Cyrtobagus salviniae* Calder and Sands) has shown the most utility as a biological control agent for managing giant salvinia (Madsen and Wersal 2009). Biological control using the weevil has been successful in tropical areas of at least 12 countries on three continents (McFarland et al. 2004). The weevils are highly host specific, favoring giant salvinia for feeding and reproduction over at least 46 other plant species (Forno et al. 1983). Adults typically reside on or beneath the fronds of giant salvinia (Center et al. 2002). The eggs are laid in cavities created by adult feeding and hatched in approximately ten days and total larvae development requires three to four weeks (McFarland et al. 2004). Adult weevils inhibit growth of the plant by consuming leaves and buds, while larvae feeding causes the fronds to turn brown and eventually drop off (Johnson et al. 2010). While the combined feeding of adults and larvae kills the plant, the main destructive factor is the larvae tunneling within the rhizomes (Sands et al. 1983). A drawback to use of salvinia weevils in the U.S., particularly northern Louisiana and Texas, is that cold winters can decimate weevil populations (Cozad et al. 2019) due to the weevil originating from tropical climates. For example, the LDWF stocked over two million salvinia weevils in Lake Bistineau from 2007 until 2015. The insects achieved no measurable success or control, even in small, closely monitored enclosures (LDWF 2016). This result is likely due in part to the inability of the weevils to survive cold winters.

Although giant salvinia can be difficult to control chemically due to its small size and ability to form thick vegetative mats (Thayer and Haller 1985), herbicides are the most widely used and effective method for controlling infestations of giant salvinia (Netherland 2014). Of the 15 active ingredients currently registered for aquatic use, ten have shown activity on giant salvinia including: bispyribac-sodium, carfentrazone-ethyl, copper, diquat dibromide, endothall, flumioxazin, fluridone, glyphosate, penoxsulam, and topramezone (Nelson et al. 2001, Glomski et al. 2003, Glomski and Getsinger 2006, Glomski and Mudge 2013, Mudge and Harms 2012, Mudge et al. 2012, 2013, Mudge 2016).

Contact herbicides such as diquat, endothall, flumioxazin, chelated copper, and carfentrazone-ethyl have shown efficacy when applied to the foliage of giant salvinia (Nelson et al. 2001, Glomski and Getsinger 2006, Glomski et al. 2003, Richardson et al. 2008). These herbicides are fast acting and can cause plant death in as few as three days (Nelson 2014); however, contact herbicides have limited ability to translocate through the plant (Lembi and Ross 1985) and only plant tissue that has come in direct contact with the herbicide is affected (Sartain 2018). This can be problematic as giant salvinia is known to create thick mats (McFarland et al. 2004), resulting in underlying plant material that can remain unaffected. Considering the rapid growth rate of giant salvinia, the untreated plants could quickly replace the plants that were damaged by contact herbicides (Sartain 2018). Systemic herbicides such as glyphosate, fluridone, bispyribac, penoxsulam, and topramezone are capable of translocating throughout the plant, but are slower acting than contact herbicides and typically require longer exposure time (Nelson 2014). Glyphosate applied to the foliage of giant salvinia results in injury after seven days and plant death by 28 days after treatment (Nelson 2014). Topramezone, penoxsulam, and bispyribac can be applied to the foliage or as a subsurface treatment, while fluridone is primarily effective as an in-water treatment (Mudge et al. 2012, Glomski and Mudge 2013, Mudge 2016). Systemic herbicides are not effective when applied subsurface to non-quiescent waters because long exposure times are required (Mudge et al. 2012). Currently, diquat, glyphosate, and a tank mix of the two are the most widely used and cost-effective herbicides used to control giant salvinia (Madsen and Wersal 2009, Mudge 2016).

1.3. Need for Additional Management Options

Due to the difficulty of chemically controlling giant salvinia, the limited number of efficacious herbicides, and the need to rotate herbicides to prevent the development of herbicide resistance, there is a need to find additional herbicides for the management of giant salvinia. In 2018, Sartain and Mudge (2018) screened several non-aquatic herbicides for activity against giant salvinia and found metsulfuron provided 98 to 100% control at 21 to 84 g active ingredient (a.i.) ha^{-1}. In addition to providing excellent control, the low rate of metsulfuron needed to control giant salvinia provides environmental benefits when compared to using high rates of glyphosate ($\geq$ 3364 g acid equivalent (a.e.) ha^{-1}) and diquat (561 g a.i. ha^{-1}).

Metsulfuron is an acetolactate synthase (ALS) inhibiting herbicide that prevents the production of the branched chain amino acids valine, leucine and isoleucine when applied to the foliage of plants or soil (Shaner 2014, University of California 2019). This herbicide inhibits plant growth within hours after application, but requires one to two weeks for injury symptoms to appear (Shaner 2014). It is labeled for use in wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), pastures, turf, right-of-way, and industrial sites to control broadleaf weeds, brush, and deciduous trees (Shaner 2014, Bayer 2019a) at 4.2 to 168.2 g a.i. ha^{-1}. Metsulfuron can also be applied in the state of Florida under the authority of a Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Special Local Need (SLN) 24(c) label to control Old World climbing fern [*Lygodium microphyllum* (Cav.) R. Br.] and for use in lake restoration projects (Bayer 2019b, Bayer 2019c). Metsulfuron has a low toxicity profile for bluegill sunfish and rainbow trout with 96-h LC_{50} (lethal concentration to kill 50% of the test population) values > 150 mg L^{-1} and > 2510 mg kg^{-1} LD_{50} (lethal dose to kill 50% of the test population) for the mallard duck (*Anas platyrhynchos* L.; Shaner 2014). ALS inhibiting herbicides generally have low toxicity to mammals, birds, fish, amphibians, etc., because the ALS enzyme is specific for plants and microorganisms (Beyer et al. 1988). Additionally, this chemistry of herbicides is approximately 100 times more toxic to plants than any herbicide in use before the 1980s (Fletcher et al. 1993). Due to this high level of phytotoxicity to target plants, extremely low foliar application rates are utilized for ALS inhibiting herbicides. The favorable toxicity profile of metsulfuron and low use rates are highly favorable from a non-target and environmental risk standpoint.

Metsulfuron is a selective herbicide, which is a desirable quality when applying chemical to areas with desired or endangered vegetation, as compared to the non-selective aquatic herbicides glyphosate and diquat. Hutchinson and Langeland (2008) demonstrated that metsulfuron has minimal effects on the native Florida plants sand cordgrass (*Spartina bakeri* Merr.), soft rush (*Juncus effuses* L.), buttonbush (*Cephalanthus occidentalis* L.), and swamp lily (*Crinum americanum* L.). Additionally, metsulfuron had no effect on torpedograss (*Panicum* *repens* L.), knotgrass (*Paspalum distichum* L.), para grass [*Brachiaria mutica* (Forssk.) Stapf], maidencane (*Panicum hemitomon* Schult.) or sawgrass (*Cladium jamaicense* Crantz) at foliar application rates up to 140 g a.i. ha^{-1} (Chiconela et al. 2004, Langeland and Link 2006). Conversely, injury did not occur in soft-stem bulrush [*Schoenoplectus tabernaemontani* (C.C. Gmel) Palla] at rates up to 70 g a.i. ha^{-1}, but growth was reduced at 140 g a.i. ha^{-1} (Chiconela et al. 2004). Metsulfuron selectivity to these plants is a highly desirable characteristic due to the importance of native vegetation in wetland and aquatic sites.

The lack of adequate aquatic herbicides to control giant salvinia has stimulated an interest in developing a SLN for metsulfuron. Following the initial evaluation periods from 2016 to 2017 (Sartain 2018, Sartain and Mudge 2018), Alligare, LLC, along with letters of support from the Louisiana Department of Wildlife and Fisheries, Texas Parks & Wildlife Department, Lower Neches Valley Authority, and the City of Shreveport requested a SLN registration in March 2019 for metsulfuron (PRO MSM 60) public water use in Louisiana and Texas to control giant salvinia (Alligare 2019). In April and July 2019, the SLN was approved by the Texas Department of Agriculture and the U.S. Environmental Protection Agency (USEPA), respectively.

Waterways are an important resource in the states of Louisiana and Texas. Unfortunately, the sub-tropical climate coupled with a long growing season provides favorable conditions for the establishment of invasive species such as giant salvinia (Sartain 2018). These states are in dire need of alternative control options for giant salvinia in order to maintain these waterways. The aforementioned desirable characteristics of metsulfuron make it a viable candidate to control giant salvinia via the SLN label. However, additional research is required to further investigate the activity of this herbicide against giant salvinia at various application rates, use patterns, as well as determine non-target species selectivity to support operational use.

Chapter 2. Evaluation of Metsulfuron for Giant Salvinia Control

2.1. Introduction

Since its introduction into the U.S. in 1995, the free-floating aquatic fern giant salvinia (*Salvinia molesta* D.S. Mitchell) has become a nuisance in lakes, rivers, and reservoirs (Johnson 1995, Jacono 1999). Giant salvinia is listed as a Federal Noxious Weed by the U.S. Department of Agriculture, which prohibits importation to the U.S. and transportation across state lines (McFarland et al. 2004). Despite these restrictions, giant salvinia has spread to 13 states since its introduction including Alabama, Arizona, Arkansas, California, Florida, Georgia, Hawaii, Louisiana, Mississippi, North Carolina, South Carolina, Texas, and Virginia (Thayer et al. 2018).

Giant salvinia is a highly invasive plant that is capable of doubling its biomass in as little as 36 hours under optimal growing conditions (Johnson et al. 2010). Due to its rapid growth rate, giant salvinia has the capability to outcompete desired native vegetation for resources such as nutrients, light, and surface area (Mitchell and Tur 1975) and can quickly form a monoculture, thus inhibiting the growth of native aquatic plants that provide food and habitat for native animals and waterfowl (Mitchell 1978). Further implications of giant salvinia infestations include, but are not limited to, hindering recreational activities such as boating, swimming, and fishing, limiting irrigation, clogging water intakes, impairing flood control, decreasing waterfront property values, and impacting rice, crawfish, and catfish farming operations (Nelson 2014, Horst and Mapes 2000).

Methods of controlling giant salvinia can be characterized as physical, biological, or chemical (Madsen and Wersal 2009). Although there are some viable physical and biological control options (Chilton et al. 2002, Madsen and Wersal 2009), chemical control through the use of aquatic herbicides is the most widely used and effective method of controlling giant salvinia

(Netherland 2014). Ten of the 15 active ingredients registered for aquatic use have demonstrated some level of activity on giant salvinia including: bispyribac-sodium, carfentrazone-ethyl, copper, diquat dibromide, endothall, flumioxazin, fluridone, glyphosate, penoxsulam, and topramezone (Nelson et al. 2001, Glomski et al. 2003, Glomski and Getsinger 2006, Glomski and Mudge 2013, Mudge and Harms 2012, Mudge et al. 2012, 2013, Mudge 2016). Currently, diquat, glyphosate, and a combination of the two are the most widely used and cost-effective treatments used to manage giant salvinia and have been used almost exclusively for controlling giant salvinia by the Louisiana Department of Wildlife and Fisheries (LDWF) (Madsen and Wersal 2009, Mudge et al. 2016, Sartain and Mudge 2018).

Due to the limited number of efficacious aquatic herbicides and the need to rotate herbicides with different modes of action to prevent the development of herbicide resistance, there is a need to find additional active ingredients to manage giant salvinia. In 2018, Sartain and Mudge (2018) screened 12 non-aquatic herbicides for activity against giant salvinia and found metsulfuron-methyl provided 98 to 100% control when applied to the foliage of plants at 21 to 84 g active ingredient (a.i.) ha^{-1}. The low use rates of metsulfuron resulted in the loss of buoyancy and desiccation of plants as early as 2 weeks after treatment (WAT) and complete control by 12 WAT when applied at 42 g a.i. ha^{-1} in the initial trial.

Metsulfuron is an acetolactate synthase (ALS) inhibiting herbicide that prevents the production of the branched chain amino acids valine, leucine and isoleucine (Shaner 2014). Metsulfuron inhibits plant growth within hours after application, but requires one to two weeks for injury symptoms to appear (Shaner 2014). It is labeled for pre- and post-emergent use in wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), pastures, turf, right-of-way, and industrial sites to control broadleaf weeds, brush and deciduous trees (Shaner 2014, Bayer

2019a) at 4.2 to 168.2 g a.i. ha^{-1}. Metsulfuron has a low toxicity profile for bluegill sunfish and rainbow trout with 96 hr LC_{50} (lethal concentration to kill 50% of the test population) values > 150 mg L^{-1} and > 2510 mg kg^{-1} LD_{50} (lethal dose to kill 50% of the test population) for the mallard duck (*Anas platyrhynchos* L.; Shaner 2014).

For many years, metsulfuron has been used to control Old World climbing fern [OWCF, *Lygodium microphyllum* (Cav.) R. Br.] in Florida and for application to dry lake beds following drawdown in state waters (Bayer 2019b, Bayer 2019c). These aquatic uses of metsulfuron in Florida have been allowed under a Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Section 24(c) registration, or Special Local Need (SLN) label. The lack of adequate aquatic herbicides to control giant salvinia has stimulated an interest in developing SLNs for that use. Following the initial evaluation periods from 2016 to 2018 (Sartain 2018, Sartain and Mudge 2018), Alligare, LLC, along with letters of support from the Louisiana Department of Wildlife and Fisheries, Texas Parks & Wildlife Department, Lower Neches Valley Authority, and the City of Shreveport requested an SLN registration in March 2019 to allow application of metsulfuron (PRO MSM 60) on public waters in Louisiana and Texas to control giant salvinia (C. Mudge Personal Communication, 2019). In April and July 2019, the SLN was approved by the Texas Department of Agriculture (TDA) and the U.S. Environmental Protection Agency (USEPA), respectively (Alligare 2019).

Although previous mesocosm trials (Sartain 2018, Sartain and Mudge 2018) demonstrated that metsulfuron is highly efficacious against giant salvinia at relatively low use rates, additional research is needed to further evaluate this herbicide at additional use rates and explore other use patterns, especially since the approval of the SLN label. Therefore, the objectives of this research were to: 1) evaluate foliar application rates to determine the most efficacious rate of metsulfuron to control giant salvinia, 2) determine if foliar applications of metsulfuron plus previously registered aquatic herbicides are compatible and if speed of injury and efficacy can be increased when applied to the foliage of plants, and 3) determine if subsurface applications of metsulfuron have activity on giant salvinia.

2.2. Materials and Methods

2.2.1 General Plant Establishment

Three outdoor mesocosm trials were conducted and repeated at the Louisiana State University (LSU) Aquaculture Research Facility in Baton Rouge, LA, in 2018 (May and July) and 2019 (May). Giant salvinia was sourced from populations maintained at the LSU Aquaculture Research Facility. For all three trials, plants in the tertiary growth stage were cultured in 76 L high density polyethylene (HDPE) containers (49.5 cm diameter by 58.4 cm height) filled with approximately 60 L of pond water (pH 8.5) amended with sphagnum moss to lower the pH (6.5 to 7.0). Equal amounts of plant material, enough to cover approximately 70% of the water surface were placed in each container. Miracle-Gro®[1] Water Soluble Lawn Food (2.1 g, 24-8-16) was added to each container at planting and periodically throughout the duration of the trials to encourage plant growth. Plants were allowed to acclimate to their new environmental conditions for two weeks before herbicide treatments were administered. At the time of treatment, plants had reached 100% coverage and were thinned to a single layer to ensure complete herbicide contact. In the subsurface titration trial, plants were approximately two layers thick since the product was applied to the water column instead of directly to the plant foliage. Planting techniques were adapted from previous giant salvinia research (Nelson et al. 2007, Mudge et al. 2012, Mudge et al. 2016, Sartain and Mudge 2018).

Treatments were arranged in a completely randomized design with 5 replicates per treatment and all trials were repeated. For all trials, biomass from 5 containers (30 total containers from 6 trials) were collected pre-treatment to measure plant biomass prior to herbicide application. In addition, a control (reference) was included in each trial to monitor plant growth in the absence of the herbicide treatments. Control plants demonstrated healthy growth with 145 to 277% increase in dry weight when compared to pre-treatment plants in all three trials (data not shown).

2.2.2. Foliar Rate Titration Trial

In the first trial, metsulfuron[2] was applied to the foliage of giant salvinia at 2.6, 5.3, 10.5, 21.1, 42.1, 84.1, and 168.2 g active ingredient (a.i.) ha^{-1} to determine the optimal use rate. All metsulfuron foliar treatments included a non-ionic surfactant[3] at 0.25% (v/v) and were applied using a forced air CO_2-powered sprayer calibrated to deliver 935 L ha^{-1} through a single TeeJet® 80-0067 nozzle[4] at 20 psi. As the herbicide was applied, a shielding device was used to minimize herbicide drift to adjacent containers/plants. At 8 weeks after treatment (WAT), all viable plant material were harvested, dried (65° C), and weighed. The dry weight data were analyzed using nonlinear regression (exponential decay, $y = b_0e^{-bx}$) with the PROC NLIN procedure in SAS®[5] version 9.4 statistical software. Regression models were used to determine the LD_{90} value, or lethal dose required to kill 90% of the test population, for metsulfuron applied to the foliage of giant salvinia. Data were pooled across trials because slopes of regression lines were not significantly different at the 95% confidence interval level.

2.2.3. Foliar Combination Trial

Herbicides evaluated in the foliar combination trial included metsulfuron alone and in combination with glyphosate[6], diquat[7], flumioxazin[8], and carfentrazone[9] (Table 2.1). Glyphosate

+ diquat was also included since this treatment is commonly used throughout the growing season (March to Nov) to control giant salvinia in Louisiana. All herbicide treatments included a non-ionic surfactant (0.25%) and were applied in the same manner as the foliar rate titration trial. Following herbicide application, visual injury (%) were documented periodically until the end of the trial. Visual estimates of giant salvinia injury were determined on a scale of 0 to 100, where 0 = no plant injury (i.e. no chlorotic/necrotic tissue) and 100 = complete plant death. At 8 WAT, all viable biomass were harvested, dried, and weighed. Dry weight data were subjected to an analysis of variance (ANOVA) and a post-HOC test (Fisher's Protected LSD) was conducted to determine significant differences among treatments ($p \leq 0.05$).

Table 2.1. Herbicide treatments applied to the foliage of giant salvinia.

Herbicide Treatment[a]	Rate (g a.i. ha^{-1})[b]
Control	0
Metsulfuron	42
Metsulfuron + Glyphosate	42 + 1121
Metsulfuron + Flumioxazin	42 + 72
Metsulfuron + Diquat	42 + 280
Metsulfuron + Carfentrazone	42 + 67
Glyphosate + Diquat	3364[c] + 561

[a] All treatments included a non-ionic surfactant at 0.25% v/v.
[b] Abbreviations: a.i., active ingredient.
[c] Glyphosate applied as g a.e. (acid equivalent) ha^{-1}.

2.2.4. Subsurface Concentration Titration Trial

In the third trial, subsurface applications of metsulfuron were administered directly to the water column at concentrations of 1, 2.5, 5, 10, 20, 40, and 80 µg a.i. L^{-1} (parts per billion, ppb) to determine if metsulfuron has activity against giant salvinia when applied to the water column. The herbicide was mixed into a 1 g L^{-1} solution with water, applied at the appropriate concentration with a pipette directly into the water column, and stirred thoroughly. Since metsulfuron in-water activity against giant salvinia was unknown, this experiment was conducted under static exposure conditions; consequently, plants were exposed to metsulfuron until the herbicide degraded, which is unknown since residue samples were not collected. At 10 WAT, all viable plant material were harvested, dried, and weighed. Plant dry weight data were analyzed using the PROC NLIN procedure, and regression models were used to determine the effective concentration 90 (EC_{90}), which is the concentration of metsulfuron required to cause a 90% reduction in dry weight compared to control plants.

2.3. Results and Discussion

2.3.1. Foliar Rate Titration Trial

In the foliar rate titration trial, metsulfuron was applied at low (2.6 g a.i. ha^{-1}) to high (168.2 g a.i. ha^{-1}) rates against giant salvinia, whereas previous research (Sartain and Mudge 2018) evaluated rates of 21, 42, and 84 g a.i. ha^{-1}. Initially, in the current trial, metsulfuron treated plants were slow to develop injury symptoms regardless of rate applied. The two lowest rates of metsulfuron (2.6 and 5.3 g a.i. ha^{-1}) resulted in visual injury symptoms by 1 WAT with stunted plant growth and minimal necrosis; however, at 2 WAT, plants treated with these low herbicide rates began to recover by the production of new juvenile buds. As the new buds developed slowly, the older, larger fronds continued to decline in health and became necrotic. By the conclusion of the trial (8 WAT), the majority of the older fronds desiccated, lost buoyancy, and fell to the bottom of the containers, leaving the stunted new growth behind.

Similar to the lower metsulfuron rates, necrosis was first observed by 1 WAT for the 10.5 to 168.2 g a.i. ha^{-1} treatments. However, throughout the duration of the trial, no plant regrowth/recovery occurred for rates $\geq$ 10.5 g a.i. ha^{-1}. By 3 WAT, all plants treated with these rates began to lose buoyancy and desiccate. At 6 WAT, the majority of the plants had fallen below the surface of the water, and by the conclusion of the trial (8 WAT), only a few necrotic, desiccated fronds remained at the surface of the water. Also, it should be noted that the highest rate tested (168.2 g a.i. ha^{-1}) did not provide faster injury symptoms or increase giant salvinia control compared to rates ≥ 10.5 g a.i. ha^{-1}.

These results confirm previous research illustrating that metsulfuron is highly efficacious against giant salvinia when applied to the foliage at very low use rates (Sartain and Mudge 2018). The calculated LD_{90} of metsulfuron when applied to the foliage of tertiary growth stage giant salvinia was 3.83 g a.i. ha^{-1} (Figure 2.1). Despite plant recovery at these low use rates, it is evident how highly active metsulfuron is against giant salvinia when applied as a foliar treatment. Similarly, metsulfuron demonstrated 100% efficacy against the highly invasive plant OWCF at 40 to 80 g a.i. ha^{-1} (Langeland and Link 2006) and is currently is used to control OWCF under an SLN label at 21 to 84 g a.i. ha^{-1} (Bayer 2019b).

The low rate of metsulfuron required to control giant salvinia, combined with its low toxicity characteristics to non-target aquatic organisms, provides environmental benefits when compared to the commonly used herbicides glyphosate [3364 g acid equivalent (a.e.) ha^{-1}] and diquat (186.6 to 560.1 g a.i. ha^{-1}), which are used at much higher use rates. Considering these benefits, operational use of metsulfuron under the SLN label should be a viable herbicide to rotate for giant salvinia management. Under the current SLN label, metsulfuron can be applied to the foliage of giant salvinia at 21 to 42 g a.i. ha^{-1} (Alligare 2019). Future field research is required to evaluate metsulfuron at these rates against plants in an operational setting to verify the small-scale results.

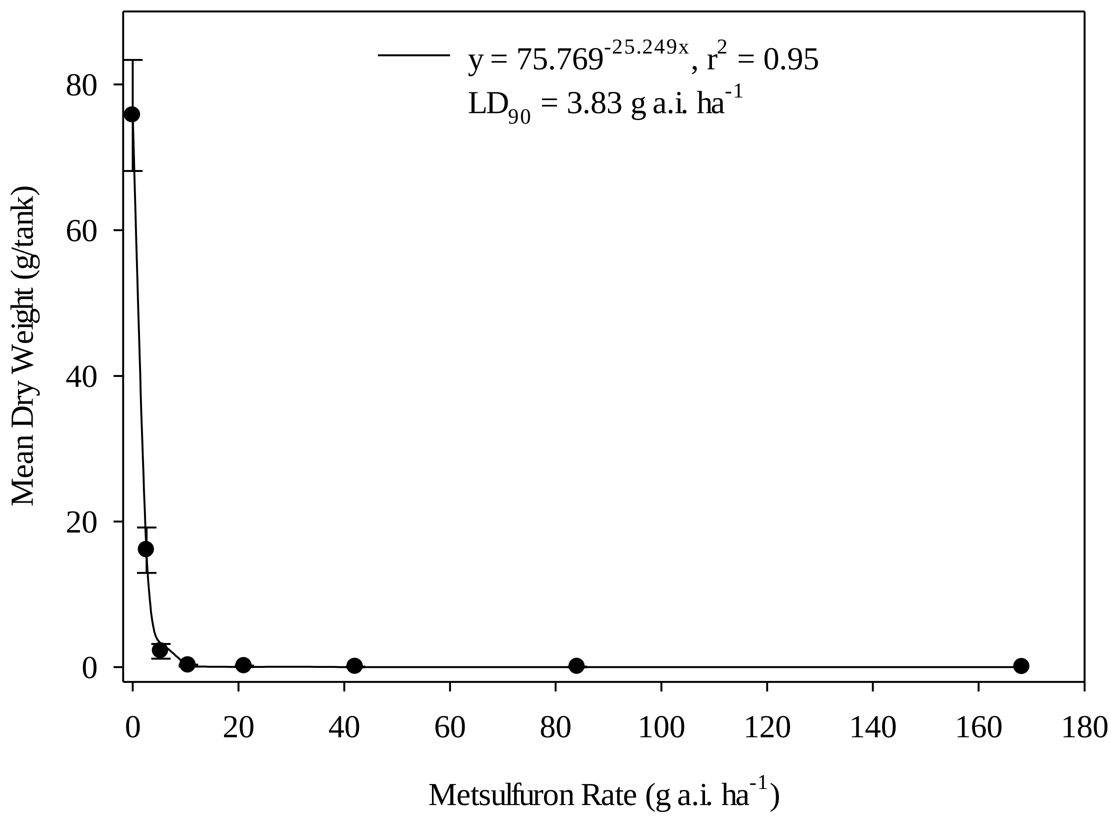

Figure 2.1. The effect of foliar applications of metsulfuron on giant salvinia dry weight 8 weeks after treatment. Data are shown as dry weight means ± standard error (n = 10). A non-ionic surfactant (0.25% v/v) was added to all treatments. LD_{90} = lethal dose 90, rate of metsulfuron required to control 90% of the giant salvinia test population. Mean pretreatment dry weight = 20.5 g.

2.3.2. Foliar Combination Trial

Similar to the rate titration trial, metsulfuron applied alone at 42 g a.i. ha^{-1} was relatively slow to injure giant salvinia when applied to the foliage (Table 2.2). Visual injury symptoms were not detectible until 1 WAT and total plant control required up to 5 weeks to achieve. In an operational setting, it would be beneficial for aquatic applicators to observe injury symptoms relatively quickly [< 24 hours after treatment (HAT)] to determine which areas have already been chemically managed.

All treatments involving metsulfuron plus carfentrazone, diquat, flumioxazin, or glyphosate resulted in ≥ 30% plant injury 1 to 3 DAT (Table 2.2). The metsulfuron +

flumioxazin and metsulfuron + carfentrazone treatments were not different than the glyphosate + diquat treatment at 3 DAT and provided faster injury than metsulfuron + glyphosate and metsulfuron + diquat. Although metsulfuron + glyphosate and metsulfuron + diquat did not provide injury as quickly as other treatments, visual injury was more substantial than metsulfuron alone (10%) at 3 DAT. However, by 2 WAT, differences among metsulfuron treatments with regard to the type and severity of plant injury were insignificant. All plants were necrotic and began to desiccate at 4 WAT, and the majority of the plants fell to the bottom of the container by the conclusion of the trial. At 8 WAT, all herbicide treatments resulted in ≥ 98% control (Figure 2.2). Despite the slow speed of metsulfuron when applied alone, there were no differences in injury and control in comparison to the combination treatments by the conclusion of the trial.

Foliar applications of glyphosate + diquat resulted in giant salvinia injury as early as 1 day after treatment (DAT) with substantial necrosis and desiccation (> 90% injury) by 2 WAT (Table 2.2). As a result, glyphosate + diquat reduced plant biomass by 96% when compared to the non-treated control at 8 WAT (Figure 2.2).

Table 2.2. Visual injury ratings (%) for giant salvinia treated with foliar applied herbicides[a]

Treatment	Rate[b]	3 DAT[c,d]	1 WAT	2 WAT	4 WAT	8 WAT
Control	0	0 a	0 a	0 a	0 a	0 a
Metsulfuron	42	10 a	50 b	70 b	90 b	98 b
Metsulfuron + Glyphosate	42 + 1121	30 b	60 bc	70 b	95 b	100 b
Metsulfuron + Diquat	42 + 280	30 b	50 b	70 b	90 b	98 b
Metsulfuron + Flumioxazin	42 + 72	40 bc	70 c	80 bc	90 b	100 b
Metsulfuron + Carfentrazone	42 + 67	40 bc	60 bc	80 bc	90 b	98 b
Glyphosate + Diquat	3364 + 561	50 c	70 c	90 c	90 b	96 b

[a] Data obtained by visually estimating giant salvinia injury on a scale of 0 to 100%, where 0 = no injury and 100 = complete plant death.
[b] Herbicides rates listed as g active ingredient (a.i.) ha^{-1}, except glyphosate, which was applied as g acid equivalent (a.e.) ha^{-1}.
[c] Abbreviations: DAT, days after treatment; WAT, weeks after treatment.
[d] Means within a column followed by the same letter are not significantly different based on Fisher's Protected LSD test ($p \leq 0.05$; $n = 10$).

These data provide evidence that the 42 g a.i. ha^{-1} rate of metsulfuron approved by USEPA and TDA is compatible when tank mixed with carfentrazone, diquat, flumioxazin, or glyphosate when applied as a foliar treatment against giant salvinia. The speed of plant injury increased and the overall level of plant control was not negatively impacted by the addition of the four herbicides to metsulfuron. These results will assist natural resource agencies with managing large population of giant salvinia, particularly when aquatic sites are continuously sprayed and applicators require site markers to find areas sprayed the previous day. Since metsulfuron requires several days to provide injury symptoms, the addition of the non-selective contact herbicides flumioxazin, carfentrazone, and diquat will not only provide faster control, but offer rapid visual markers (hours to 1 day), which can aid in distinguishing treated vs. untreated sites. Combining the non-selective systemic herbicide glyphosate with metsulfuron resulted in slower injury than the contact herbicides (3 days), but still faster than metsulfuron alone. Previous research (Mudge and Netherland 2014, Mudge and Netherland 2015) demonstrated a similar concept when the slow-acting ALS herbicides penoxsulam, imazamox, and bispyribac were combined with several contact herbicides to provide rapid injury and control to water hyacinth [*Eichhornia crassipes* (Mart.) Solms] and waterlettuce (*Pistia stratiotes* L.).

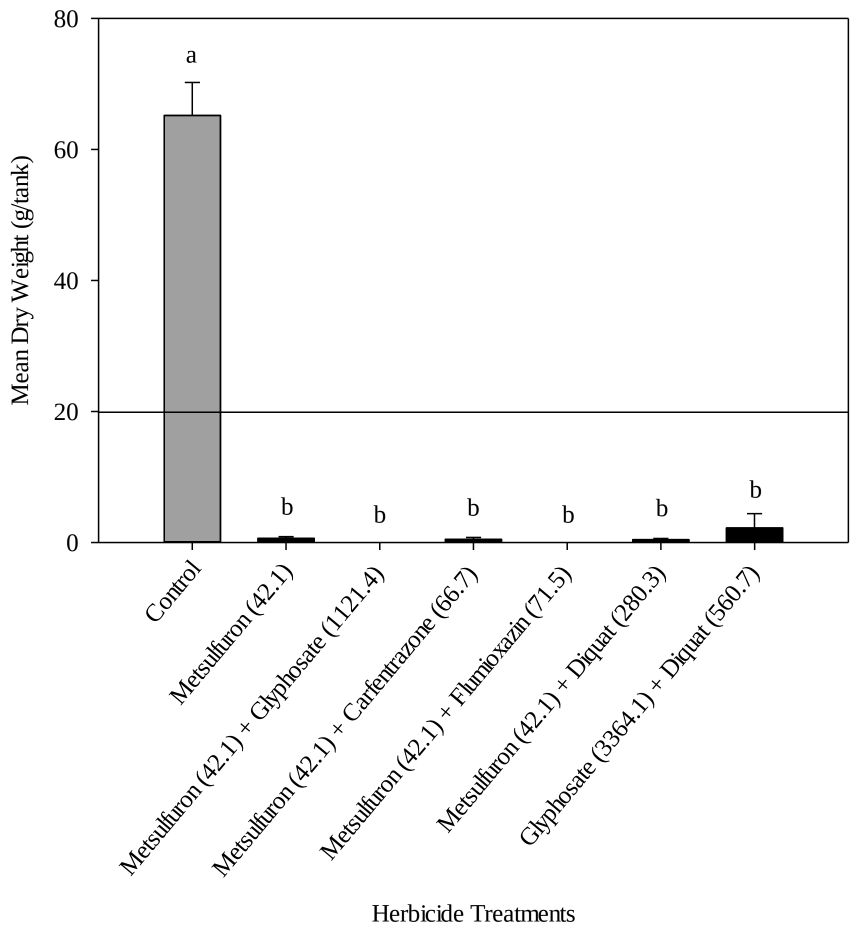

Figure 2.2. The effect of foliar herbicide applications on giant salvinia dry weight (mean ± standard error) at 8 weeks after treatment. Numbers behind herbicides represent herbicide rates in g a.i. ha^{-1}, except glyphosate, which was applied as g acid equivalent (a.e.) ha^{-1}. A non-ionic surfactant (0.25% v/v) was added to all treatments. Treatments sharing the same letter are not significant according to Fisher's Protected LSD ($p \leq 0.05$; $n = 10$). Horizontal line represents mean pre-treatment dry weight.

2.3.3. Subsurface Concentration Titration Trial

Similar to the foliar rate titration trial, visual injury was very slow to develop for giant salvinia treated subsurface with metsulfuron. For the lowest 3 concentrations of metsulfuron evaluated (1, 2.5 and 5 µg a.i. L^{-1}), visual injury (necrosis) was not observed until 2 WAT, but growth was halted by 1 WAT. Plants exposed to metsulfuron at ≤ 5 µg a.i. L^{-1} became increasingly necrotic from 2 to 3 WAT; however, at 4 WAT, new buds began to form. By the conclusion of the trial, the majority of the herbicide injured fronds/plants exposed to these concentrations sunk to the bottom of the containers and the plants were healthy, actively growing, and similar in appearance to the control plants.

Visual injury was 10 to 20% at 1 WAT for plants exposed to metsulfuron at 10 to 80 µg a.i. L^{-1}. At 2 WAT, these same plants exhibited a significantly higher amount of visual injury (> 50% injury) compared to the lower concentrations. Unlike the lower concentration treatments, no plant recovery occurred at doses ≥ 10 µg a.i. L^{-1}. Plants were initially necrotic, began to lose buoyancy at 4 WAT, and continued to decline in health during the remainder of the trials. At the conclusion of the trial (10 WAT), > 98% of the plant material fell below the water surface with only a few desiccated, unhealthy fronds remaining at the surface of the water in the containers treated with 10 to 80 µg a.i. L^{-1} metsulfuron.

The calculated EC_{90} for metsulfuron applied subsurface against giant salvinia was 1.87 µg a.i. L^{-1} (Figure 2.3). Similar to the metsulfuron foliar rate titration results, plant regrowth would occur at this low concentration, but it can be concluded that metsulfuron is highly active as an in-water treatment against giant salvinia in the tertiary growth stage when plants are cultured in water with a pH ranging from 6.5 to 7.0. Metsulfuron has also shown activity against the target aquatic plants common duckweed (*Lemna minor* L.) and Eurasian watermilfoil

(*Myriophyllum spicatum* L.), with EC_{50} values of 0.36 and 0.816 μg a.i. L^{-1}, respectively (EPA 2019a, 2019b). The high level of efficacy at extremely low concentrations against these target plants is a favorable characteristic for aquatic use. The current data as well as previous findings (EPA 2019a, 2019b) could be used as supporting material if an herbicide registrant were considering applying for a FIFRA Section 3 aquatic label for metsulfuron use in the U.S. However, additional research would be required for this method of application to be utilized in an operational setting. Since these experiments were conducted under static conditions, future research should investigate the impact of concentration exposure times (CET) to determine how active metsulfuron is when applied subsurface under various CET regimes. The effect of water pH on in-water activity of metsulfuron should also be investigated since sulfonylurea herbicides are degraded at faster rates by acid hydrolysis, which increases at lower pH levels (Grey and McCullough 2012). Metsulfuron half-life ranges from 4 to 9.6 days at pH 5.2 and 116 days at pH 7.1 (National Center for Biotechnology Information 2019). Since giant salvinia thrives at a pH < 7.5 (Cary and Weerts 1984) and has the ability to decrease the pH over time, metsulfuron should degrade relatively quickly in waterbodies throughout Louisiana and Texas. A short herbicide half-life is favorable from an environmental standpoint, but could result in decreased efficacy at lower pH levels due to faster degradation of the compound.

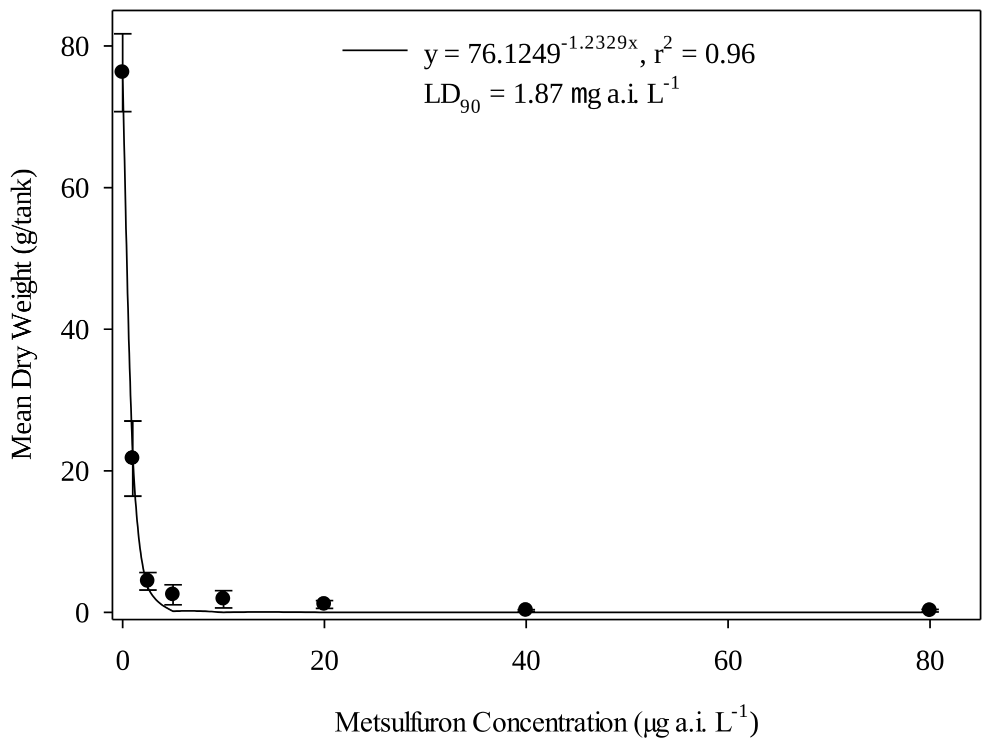

Figure 2.3. The effect of subsurface applications of metsulfuron on giant salvinia dry weight 10 weeks after treatment. Metsulfuron was applied as a single application and the plants were exposed to the herbicide under static conditions. Data are shown as dry weight means ± standard error (n = 10). EC90 = effective concentration 90, concentration of metsulfuron required to control 90% of the giant salvinia test population. Mean pretreatment dry weight = 40.2 g.

The results of these three experiments demonstrate that metsulfuron is highly active against giant salvinia when applied to the foliage or subsurface at a low rate or concentration. When applied to the foliage alone, metsulfuron treated plants were slow to develop visual injury symptoms, but provided > 98% plant control by 8 WAT at rates of 10.5 to 168.2 g a.i. ha^{-1}. Metsulfuron was also compatible in tank mix combinations with glyphosate, flumioxazin, diquat, and carfentrazone. The addition of these herbicides would not be necessary to control the plant since metsulfuron alone provided > 98% control at rates ≥ 10.5 g a.i. ha^{-1}, but tank mix combinations increased speed of injury, which would provide a rapid visual marker for aquatic applicators. Subsurface applications of metsulfuron were also highly efficacious, and provided > 98% control by 10 WAT at concentrations of 10 to 80 µg a.i. L^{-1}. The high level of giant salvinia activity as a foliar and subsurface treatment further supports previous research (Sartain and Mudge 2018), indicating that metsulfuron is a viable control option for this highly invasive floating fern.

2.4. Sources of Materials

[1] Miracle-Gro® Lawn Fertilizer, The Scotts Company, P.O. Box 606 Marysville, Ohio 43040.

[2] MSM 60® Alligare, LLC, 13 N. 8th Street, Opelika, AL 36801.

[3] Surf-AC® 910, Drexel Chemical Company, P.O. Box 13327, Memphis, TN 38113.

[4] TeeJet®, Spraying Systems Co., P.O. Box 7900 Wheaton, IL 60187.

[5] SAS® software version 9.4, SAS Institute Inc., 100 SAS Campus Drive Cary, NC 27513.

[6] Roundup Custom™, Bayer CropScience LP, P.O. Box 12014, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709.

[7] Reward®, Syngenta Crop Protection LLC. P.O. Box 18300, Greensboro, NC 27419.

[8] Clipper®, Valent U.S.A. Corporation, P.O. Box 8025. Walnut Creek CA 94596.

[9] Stingray®, SePRO Corporation, 11550 N. Meridian St., Suite 600, Carmel, IN. 46032.

Chapter 3. Evaluation of Non-Target Aquatic and Terrestrial Plant Species Response to Metsulfuron

3.1. Introduction

The invasive aquatic fern, giant salvinia (*Salvinia molesta* D.S. Mitchell) has continued to invade waterbodies across the southern U.S. since its introduction in 1995 (Johnson 1995). Giant salvinia exhibits an extremely rapid growth rate, and is capable of doubling its biomass in as few as 36 hours under ideal growing conditions (Johnson et al. 2010). Due to this rapid growth rate, giant salvinia can quickly outcompete native vegetation and create a monoculture (Mitchell and Tur 1975). Infestations can have negative impacts including, but not limited to, decreased wildlife habitat and water quality, disruption of transportation, irrigation, and recreational activities, lowered property values, mosquito breeding habitat, and public health concerns (Jacono 1999, Jacono and Pittman 2001, Nelson et al. 2001).

Currently, glyphosate and diquat are the most utilized herbicides in Louisiana for giant salvinia management (Mudge et al. 2016), but other herbicides including carfentrazone-ethyl, flumioxazin, bispyribac-sodium, penoxsulam, and topramezone have demonstrated varying levels of control when applied alone or in combination with other chemistries (Glomski and Getsinger 2006, Mudge and Harms 2012, Mudge et al. 2012, Glomski and Mudge 2013, Mudge 2016, Mudge et al. 2016). Due to the difficulty in chemically controlling giant salvinia, the limited number of efficacious herbicides registered for aquatic sites, and the need to rotate herbicides to prevent the development of herbicide resistance, there is a need for additional herbicide options to control giant salvinia (Sartain and Mudge 2018). Previous research has shown that metsulfuron provided 98 to 100% control of giant salvinia at low use rates [21.1 to 84.1 g active ingredient (a.i.) ha^{1}] when applied to the foliage of the plant (Sartain and Mudge 2018).

Metsulfuron is an acetolactate synthase (ALS) inhibiting herbicide that is labeled for use in wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), pastures, turf, right-of-way, and industrial sites to control broadleaf weeds, brush, and deciduous trees (Shaner 2014, Bayer 2019a) at 4.2 to 168.2 g a.i. ha^{-1}. In addition, metsulfuron can be applied in the state of Florida under the authority of a Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Special Local Need (SLN) 24(c) label to control Old World climbing fern [*Lygodium microphyllum* (Cav.) R. Br.] and for use in dewatered zones of lakes (Bayer 2019b, c).

Metsulfuron has a low toxicity profile for bluegill sunfish and rainbow trout with 96 hr LC_{50} (lethal concentration to kill 50% of the test population) values > 150 mg L^{-1} for both species and > 2510 mg kg^{-1} LD_{50} (lethal dose to kill 50% of the test population) for the mallard duck (*Anas platyrhynchos* L.; Shaner 2014). Metsulfuron degrades by acid hydrolysis (Grey and McCullough 2012), and at the lower pH threshold where giant salvinia thrives (Cary and Weerts 1984), hydrolysis is likely to occur more rapidly and degradation is to occur relatively quickly.

A primary objective of aquatic weed management is to control growth of invasive plant species while maintaining a diversity of native species (Mudge and Haller 2010). Native aquatic plants can improve water clarity and quality, provide valuable fish and wildlife habitat, reduce sediment resuspension, and help prevent the spread of invasive plants (Savino and Stein 1982, Heitmeyer and Vohs 1984, Smart 1995, Dibble et al. 1996). However, damage to native species can result from subsurface and foliar applications of herbicides and is an important factor in herbicide selection (Mudge and Haller 2010). Metsulfuron is a selective herbicide, which is a desirable quality when applying chemicals to areas with desired or endangered vegetation, whereas glyphosate and diquat are considered non-selective herbicides (Shaner 2014, Nufarm 2019). Previous research demonstrated that metsulfuron had minimal effects on the aquatic plants sand cordgrass (*Spartina bakeri* Merr.), soft rush (*Juncus effuses* L.), maidencane (*Panicum hemitomon* Schult.), sawgrass (*Cladium jamaicense* Crantz.), and buttonbush (*Cephalanthus occidentalis* L.) at foliar application rates up to 168 g a.i. ha^{-1} (Hutchinson and Langeland 2008, Langeland and Link 2006). Conversely, Hutchinson and Langeland (2008) and Chiconela et al. (2004) reported that foliar applications of metsulfuron ≤ 168 g a.i. ha^{-1} severely impacted lizard's tail (*Saururus cernuus* L.), golden canna, (*Canna flaccida* Salisb.) fireflag (*Thalia geniculata* L.), swamp fern (*Blechnum serrulatum* Rich.), pickerelweed (*Pontederia cordata* L.) and arrowhead (*Sagittaria lancifolia* L.). Since limited information is available regarding sensitivity of aquatic plants to metsulfuron, additional research is needed to further evaluate non-target species susceptibility to metsulfuron.

In April and July 2019, a SLN label was approved by the Texas Department of Agriculture (TDA) and the U.S. Environmental Protection Agency (USEPA), respectively, allowing the foliar application of metsulfuron to control giant salvinia on public water in Texas (Alligare 2019). As the use of this herbicide in aquatic sites increases, it is important to determine the effects of foliar applied metsulfuron on common non-target species that occur in or near areas where giant salvinia has infested.

Additionally, it is common for homeowners, commercial nurseries, and farmers to source irrigation water from nearby waterbodies (Hodges and Haydu 2006). Non-target terrestrial species can be negatively impacted if waters treated with herbicides for aquatic weed control are used for irrigation (Mudge and Haller 2009). The phytotoxic effects of irrigation water containing the aquatic herbicides 2,4-D, bispyribac, copper, flumioxazin, fluridone, diquat, endothall and topramezone have been investigated on non-target turf, ornamental, and vegetable species (Hiltibran and Turgeon 1977, Reimer and Motto 1980, Andrew et al. 2003, Koschnick et al. 2005a, 2005b, Mudge et al. 2007, Mudge and Haller 2009, Gettys and Haller 2012, Haller et al. 2017), but have not been investigated with metsulfuron. The USEPA evaluates the impacts and risks associated with aquatic herbicides in water and may impose water use restrictions on treated water to protect human health and the environment. Tolerances of metsulfuron on certain food crops have been established by the USEPA by determining the maximum amount of pesticide residue that can remain in or on a treated food commodity to ensure food safety (EPA 2017), but no such tolerances are required for ornamental plants (nonfood crops). While higher priority is placed on food crops, the potential effects of using herbicide treated water to irrigate ornamentals and other plants not destined for human consumption are still a concern (Haller et al. 2017). Therefore, when water treated with an aquatic herbicide is used for irrigation of both food and nonfood crops and herbicide residues remain, phytotoxicity is a concern (Mudge and Haller 2009). Research was conducted to 1) determine the sensitivity of non-target aquatic species to foliar applied metsulfuron and 2) determine the phytotoxic effects of metsulfuron treated irrigation water on non-target aquatic and terrestrial species.

3.2. Materials and Methods

Outdoor mesocosm experiments were conducted in April and June 2019 at the Louisiana State University (LSU) Aquaculture Research Facility in Baton Rouge, Louisiana to determine the selectivity of foliar applications of metsulfuron on non-target aquatic plants including giant blue iris (*Iris giganticaerulea* Small.), broadleaf arrowhead (*Sagittaria latifolia* Willd.), yellow water lily (*Nymphaea mexicana* Zucc.), and broadleaf cattail (*Typha latifolia* L.). In addition, outdoor mesocosm trials were conducted in April and June 2019 to evaluate the susceptibility of the non-target aquatic giant blue iris, and terrestrial plants cherry tomato (*Solanum lycopersicum* L. 'Sun Gold'), vinca [*Catharanthus roseus* (L.) G. Don. 'Pacifica Cherry Halo'], and soybean

[*Glycine max* (L.) Merr. 'Asgrow® 5535'] to metsulfuron in irrigation water. All non-target aquatic and terrestrial species evaluated in the metsulfuron foliar and irrigation trials are commonly found throughout Louisiana.

3.2.1. Foliar Applied Metsulfuron vs. Non-target Aquatic Species

Giant blue iris plants were purchased from a commercial grower in Louisiana[1] at a height of approximately 25 cm and grown in 2.5 L high density polyethylene (HDPE) pots (14.0 cm diameter by 16.4 cm height) as purchased from the grower. Each pot contained 3 or 4 iris rhizomes with a commercial potting medium and top-dressed with a slow-release fertilizer[2] (15-9-12) at a rate of 2 g kg^{-1} soil upon arrival. Experimental plants were selected on the basis of uniform height to minimize variation. Plants were placed in trays (69 cm x 53 cm x 16 cm) maintained with 15 cm of water and allowed to acclimate for two weeks before herbicide treatments were administered. Plants were approximately 33 cm tall and actively growing with no floral production at the time of treatment in both the foliar and irrigation trials.

Broadleaf arrowhead and yellow water lily were obtained from a commercial grower in Florida[3] as bare root plants and cattail were collected from ponds at the LSU Aquaculture Research Facility. Broadleaf arrowhead and yellow water lily were transferred into 2.5 L HDPE pots, while cattail were transferred into 14 L HDPE pots to accommodate their substantial rhizome biomass. All pots contained a commercially available topsoil[4] and slow-release fertilizer at a rate of 2 g kg^{-1} soil. A 2.5 cm layer of masonry sand was added to the top of the soil to limit nutrient exchange with the water column. Pots were then placed in 1135 L tanks filled with pond water (pH 8.5) to a height of 10 cm for broadleaf arrowhead and cattail, and 45 cm for yellow water lily. As broadleaf arrowhead and cattail elongated water level was increased slowly until a final height of 45 cm was achieved. All three species were allowed to acclimate to their new environment for six weeks before being individually transferred to 76 L high density polyethylene (HDPE) containers (49.5 cm diameter by 58.4 cm height) filled to 45 cm with pond water for the experimentation phase of the trial. All plants selected for experimentation were uniform in biomass at the time of treatment.

All plants were arranged using a completely randomized design with 5 replicates per treatment for iris, 4 replicates for cattail and broadleaf arrowhead, and 3 replicates for yellow water lily due to plant availability. All trials were conducted and repeated in 2019. Biomass was collected pre-treatment as a reference to determine plant growth throughout the trial and a non-treated control was included to monitor plant growth in the absence of herbicide treatments.

Metsulfuron[5] was applied to the foliage of all species at 10.5, 21.1, 42.1, and 84.1 g a.i. ha^{-1} using a forced air CO_2-powered sprayer calibrated to deliver the equivalent of 935 L ha^{-1} diluent through a single TeeJet® 80-0067 nozzle[6] at 20 psi. All treatments included a non-ionic surfactant[7] (0.25% v/v). As the herbicide was applied, a shielding device was used to minimize herbicide drift to adjacent plants. To evaluate species susceptibility to metsulfuron, all viable plant material were harvested at 7 weeks after treatment (WAT) and dried in an oven (65° C) to constant weight. Dry weight data from each trial were subjected to an analysis of variance (ANOVA) using SAS®[8] version 9.4 statistical software. A post-HOC test (Fisher's Protected LSD) was then conducted to determine significant differences among treatments ($p \leq 0.05$). Data were pooled across experimental runs for all species since no trial interactions were detected.

3.2.2. Metsulfuron Irrigation vs. Non-target Species

Giant blue iris plants were cultivated and maintained in the same manner as the previous experiment. Cherry tomato and vinca were obtained as 5 cm starter plants from the same commercial nursery as the irises and soybean seeds were obtained from the LSU Central Research Station. Cherry tomato and vinca plants were immediately transplanted, and three soybean seeds per pot were planted into 2.5 L HDPE pots containing topsoil and slow-release fertilizer at a rate of 2 g kg^{-1} soil. Once soybean seeds germinated, plants were thinned to two plants per pot. When the metsulfuron irrigation treatments were applied, cherry tomato and vinca plants were actively growing, beginning to flower, and relatively uniform in size and biomass. Soybeans were treated when all plants reached the V5 to V6 growth stage (vegetative growth with 5 to 6 unfolded trifoliate leaves). Cherry tomato, vinca, and soybean plants remained in an outdoor mesocosm environment under full sunlight for the duration of the trial and were irrigated with ca. 1.27 cm of city water daily using an overhead sprinkler system on an automated timer.

All species were arranged in a completely randomized design with five replicates per treatment and the trials were repeated. Biomass was collected pre-treatment and a non-treated control was included for all species to monitor plant growth in the absence of herbicide treatments. Plants were overhead irrigated once using a watering can with 1.27 cm metsulfuron treated water at concentrations of 1, 2.5, 5, 10, 20, and 40 µg a.i. L^{-1}. This amount of irrigation water was sufficient to cover the plants and saturate the soil. Metsulfuron was mixed into a 1 g L^{-1} stock solution, pipetted directly into the watering can, mixed thoroughly with city water, and immediately applied to the plants. The purpose of these treatments was to simulate an irrigation event with water sourced from a waterbody recently treated with various concentrations of metsulfuron. At 24 hours after treatment (HAT), the previous irrigation schedule resumed for the terrestrial species with herbicide-free water for the remainder of the trial.

Since cherry tomato plants began to produce mature fruit at ca. 2 WAT, five mature fruit were harvested from each replicate at 4 and 5 WAT. Fresh weight of these fruit were immediately recorded to determine the impact of metsulfuron on cherry tomato fruit production. All viable aboveground biomass (stems, leaves, flowers, fruit, seed pods, etc.) were harvested 7 WAT for iris and 6 WAT for cherry tomato, vinca, and soybean. Plants were then dried in an oven (65° C) for 7 days and weighed. Dry weight data for all species and cherry tomato fruit fresh weight were subjected to the same statistical procedures as the metsulfuron foliar non-target trial. Data for all species were pooled across experimental runs as there was no trial interactions detected.

3.3. Results and Discussion

3.3.1. Foliar Applied Metsulfuron vs. Non-target Aquatic Species

Giant blue irises were highly sensitive to all foliar application rates of metsulfuron evaluated. Herbicide symptoms were relatively slow to develop, which is a typical trait of metsulfuron (Shaner 2014). At 2 WAT, all herbicide treated plants were stunted as plant height was 2 to 4 cm shorter than the control plants (data not shown). At 3 WAT, control plants were actively growing and beginning floral production, while herbicide treated plants were chlorotic and no flowers were observed. After this point forward, metsulfuron treated plants became increasingly necrotic. At the conclusion of the experiment (7 WAT), control plant biomass had increased by 160% since herbicide treatments were administered. At 7 WAT, dry weight of the irises were reduced 97 to 99% when metsulfuron was applied to the foliage of the plants (Table 3.1) with no observable regrowth from above or belowground plant appendages.

Dissimilar to other species evaluated in the foliar research trials, herbicide symptomology occurred quickly for broadleaf arrowhead treated with metsulfuron. Plants exhibited stunted growth and necrosis by 3 DAT, with total plant death occurring by 2 WAT. At 7 WAT, all foliar applied herbicide treatments resulted in 100% reduction in biomass, while control plants were still actively growing and had substantially increased in biomass compared to pre-treatment weights (Table 3.1).

Yellow water lily plants were flowering and vegetative growth was slowing at the time of treatment. As a result of this halted growth, control plants exhibited limited difference in biomass from pre-treatment to harvest; however, plants were still healthy and covered 100% of the water surface 7 WAT. Since the plants were mature at herbicide application, there was an approximate 15% overlap of the surface leaves, thus 100% herbicide contact with all leaves was not obtained. Although this plant architecture is not ideal when managing target species, this situation can occur during an operational scale treatment. At 3 WAT, leaves that had come in direct contact with the herbicide spray solution were desiccated, while injury was limited for leaves not contacted by the herbicide solution. However, for the remainder of the trial, plant injury increased and the herbicide continued to translocate through the plant, slowly affecting emergent and underwater tissue (i.e., leaves and stems) that were not originally and directly contacted by the herbicide application. At 7 WAT, no regrowth had occurred, plant appendages were continuing to decline in health, and remnant leaves were desiccating. Metsulfuron treated plants were reduced 84 to 87% in dry weight compared to the control, and there were no differences among the herbicide treatments (Table 3.1).

Cattail was the only species in which an herbicide rate effect was detected. Visually, plant height and biomass increased throughout the experiment for plants treated with metsulfuron at 10.5 and 21.1 g a.i. ha^{-1}, but plant growth and development was slower than the non-treated control. Conversely, plants treated with 42.1 and 84.1 g a.i. ha^{-1} displayed stunted growth and necrosis by 2 WAT. These plants became increasingly necrotic throughout the remainder of the trial and no regrowth occurred. At 7 WAT, there were no significant differences in dry weight biomass between the control and the metsulfuron treatments of 10.5 and 21.1 g a.i. ha^{-1}, whereas the 42.1 and 84.1 g a.i. ha^{-1} treatments reduced plant dry weight by 85 and 91%, respectively (Table 3.1).

Table 3.1. The effect of foliar applied metsulfuron on 4 non-target aquatic plant species 7 weeks after treatment[a].

Metsulfuron Rate (g a.i. ha^{-1})[b]	Dry Weight (g ± SE)			
	Giant Blue Iris[c]	Broadleaf Arrowhead	Yellow Water Lily	Broadleaf Cattail
0	31.5 ± 1.8 a	14.4 ± 1.5 a	23.7 ± 1.5 a	33.6 ± 2.2 a
10.5	0.7 ± 0.5 b	0.0 ± 0.0 b	3.7 ± 0.6 b	27.1 ± 4.0 a
21.1	0.2 ± 0.2 b	0.0 ± 0.0 b	3.0 ± 0.4 b	24.4 ± 4.9 a
42.1	0.3 ± 0.2 b	0.0 ± 0.0 b	3.1 ± 0.5 b	4.7 ± 2.5 b
84.1	0.8 ± 0.4 b	0.0 ± 0.0 b	2.9 ± 0.7 b	2.8 ± 1.9 b

[a] Pre-trial dry weight (g ± SE): giant blue iris, 12.1 ± 0.8; broadleaf arrowhead, 11.3 ± 1.2; yellow water lily, 22.7 ± 1.1; cattail, 21.4 ± 2.6.
[b] Abbreviations: a.i., active ingredient; SE, standard error.
[c] Means within a column followed by the same letter are not significantly different based on Fisher's Protected LSD test ($p \leq 0.05$; $n = 10$).

Based on these data, giant blue iris, broadleaf arrowhead, yellow water lily, and broadleaf cattail are sensitive to foliar applications of metsulfuron at 10.5 to 84.1 g a.i. ha^{-1}. Rates ≥ 10.5 g a.i. ha^{-1} resulted in complete plant death of iris and broadleaf arrowhead, as well as 84 to 87% reduction of yellow water lily biomass. Despite cattail not being impacted by foliar application rates ≤ 21.1 g a.i. ha^{-1}, the higher rates (42.1 and 84.1 g a.i. ha^{-1}) resulted in 85 to 91% control.

If giant salvinia were treated with metsulfuron as a foliar application, non-target aquatic plants growing in the vicinity could be negatively impacted. Although giant salvinia infestations typically exist as a monoculture (Mitchell 1978), it is important to understand the effect of any herbicide application on other species that may be growing in the vicinity. At the foliar use rate of 42.1 g a.i. ha^{-1} approved by the SLN label in Texas and possible future use in Louisiana, the results generated under mesocosm conditions suggest that giant blue iris, broadleaf arrowhead, yellow water lily, and broadleaf cattail would all be significantly injured or killed by treatments targeting giant salvinia. In aquatic weed management, one of the primary goals is to selectively control the target plant(s) while minimizing the impacts on the desirable non-target/native plant community. Since limited information is available regarding sensitivity of aquatic plants to metsulfuron, the results of this experiment, combined with previous research (Hutchinson and Langeland 2008, Chiconela et al. 2004, Langeland and Link 2006), provide valuable information to natural resource agencies who will use metsulfuron under the SLN label to manage giant salvinia. If any of the non-target species evaluated in the current and previous research (Hutchinson and Langeland 2008, Chiconela et al. 2004, Langeland and Link 2006) are present, aquatic applicators should adjust their spray techniques and application methods to minimize damage to desirable plant species.

3.3.2. Metsulfuron Irrigation vs. Non-target Species

Giant blue iris was the most sensitive plant to irrigation water treated with metsulfuron. The non-treated control plants exhibited a 147% increase in biomass compared to pre-treatment biomass, thus indicating healthy growth throughout the trial (Table 3.2). By the conclusion of the trial (7 WAT), all plants, regardless of treatment, had flowered at some point during the trial and were beginning to decline in health. There were no differences in plant dry weight between the control and the metsulfuron irrigation treatments of 1, 2.5, 5, and 10 µg a.i. L^{-1} (Table 3.3). Plants treated with metsulfuron at 20 µg a.i. L^{-1} exhibited slight discoloration and reduced flowering throughout the trial. However, biomass in the 40 µg a.i. L^{-1} treatment was significantly different from all treatments except for the 20 µg a.i. L^{-1} treatment. Despite the 29% reduction in dry weight compared to control, irises irrigated with the 40 µg a.i. L^{-1} treatment exhibited a 73% increase in biomass when compared to the pre-treatment plants and were still actively growing and flowering at the time of harvest.

Metsulfuron applied in irrigation water had no impact on the cherry tomato plants (Table 3.2). After herbicide treatments were administered, plants continued to grow with no adverse visual effects. At ca. 2 WAT, plants across all treatments began to produce healthy fruit. In addition, there were no differences in fruit fresh weight across all treatments at 4 and 5 WAT (Table 3.2). When the cherry tomatoes were harvested at 6 WAT, plants were actively growing, exhibiting normal fruit production, and biomass of all treatments increased 677 to 766% from pre-treatment to final harvest.

Table 3.2. The effect of a single overhead irrigation with 1.27 cm water containing metsulfuron on cherry tomato fruit fresh weight and plant dry weight.

Metsulfuron Concentration (µg a.i. L^{-1})[a]	Cherry Tomato Fruit Fresh Weight (g ± SE)		Cherry Tomato[b] Dry Plant Weight (g ± SE)
	4 WAT	5 WAT	6 WAT
0	10.0 ± 0.9	11.8 ± 0.8	18.8 ± 0.6
1	10.5 ± 1.2	11.8 ± 0.8	18.8 ± 0.8
2.5	13.0 ± 1.5	14.1 ± 0.8	18.5 ± 0.9
5	10.3 ± 0.9	11.7 ± 1.3	18.0 ± 0.9
10	12.1 ± 1.8	14.1 ± 0.7	20.1 ± 0.8
20	12.3 ± 0.7	13.5 ± 0.8	19.5 ± 1.0
40	12.2 ± 1.6	12.5 ± 1.0	18.1 ± 0.8

[a] Abbreviations: a.i., active ingredient; SE, standard error; WAT, weeks after treatment.
[b] Pre-treatment dry weight (g ± SE) of plants: 2.3 ± 0.2.

Similar to the cherry tomato trial, there were no differences in vinca dry weight biomass across all treatments (Table 3.3). Plants continued growing normally after herbicide treatments were applied until plants were harvested with no noticeable herbicide symptomology and all control and metsulfuron irrigated plants produced normal, abundant flowers. At the conclusion of the experiment, plant biomass increased in all treatments (control and metsulfuron) by 331 to 413% when compared to the pre-treatment level.

After metsulfuron treatments (1 to 40 µg a.i. L^{-1}) were administered via overhead irrigation to soybeans in the V5 to V6 growth stage, plants continued to mature normally and transitioned into the reproductive growth stage within 7 DAT. At the time of harvest (6 WAT), all plants were healthy and producing seed pods. There were no differences in plant dry weight among the metsulfuron treatments; however, dry weight was significantly lower for the control compared to the metsulfuron irrigated plants (Table 3.3). Herbicide irrigated plant biomass increased by 1214 to 1338% when compared to pre-treatment biomass, while the non-treated plant biomass increased 937%.

Table 3.3. The impact of a single overhead irrigation with 1.27 cm water containing metsulfuron on the dry weight of non-target aquatic and terrestrial plants at 6 (vinca and soybean) and 7 (iris) weeks after treatment[a].

Metsulfuron Concentration (µg a.i. L^{-1})[b]	Dry Weight (g ± SE)		
	Giant Blue Iris[c]	Vinca	Soybean
0	27.2 ± 2.7 a	6.8 ± 0.5	21.7 ± 1.6 b
1	27.5 ± 1.1 a	5.5 ± 0.6	29.5 ± 1.3 a
2.5	26.1 ± 3.3 a	5.3 ± 0.4	27.7 ± 2.7 a
5	28.3 ± 2.5 a	6.1 ± 0.8	29.5 ± 2.1 a
10	27.7 ± 2.3 a	6.3 ± 0.6	28.9 ± 2.1 a
20	25.6 ± 1.7 ab	6.2 ± 0.4	30.1 ± 1.8 a
40	19.3 ± 2.1 b	5.9 ± 0.6	27.5 ± 2.3 a

[a] Pre-trial dry weight (g ± SE): giant blue iris, 11.2 ± 0.4; vinca, 1.2 ± 0.1; soybean, 2.1 ± 0.1.
[b] Abbreviations: a.i., active ingredient; SE, standard error.
[c] Means within a column followed by the same letter are not significantly different based on Fisher's Protected LSD test ($p \leq 0.05$; $n = 10$).

These data provide evidence that the varieties of cherry tomato, vinca, and soybean evaluated in this research were not negatively impacted by irrigation water containing metsulfuron at concentrations ranging from 1 to 40 µg a.i. ha^{-1}. The plants in these research trials were relatively young at the time of treatment and it is speculated that mature plants will also likely be tolerant. Although irises were unaffected by concentrations ≤ 20 µg a.i. L^{-1}, metsulfuron applied at 40 µg a.i. L^{-1} stunted plant growth and reduced dry plant biomass by 29% compared to the control. It should also be noted that in the metsulfuron foliar non-target trial, irises were highly sensitive to metsulfuron applied to the foliage at rates as low as 10.5 g a.i. ha^{-1} (Table 3.1). However, plants were much more tolerant to metsulfuron applied via irrigation water (Table 3.3). Besides the rate/concentration differences between trials (10.5 to 84.1 g a.i. ha^{-1} vs. 1 to 80 µg a.i. L^{-1}), this could be resultant of the smaller water droplets produced by the TeeJet 80-0067 spray nozzle compared to the larger droplets exiting the watering can, or the non-ionic surfactant included in the foliar application. The function of a surfactant is to improve herbicide contact and absorption by physically modifying the deposition and wetting characteristics of the spray solution (Winfield 2019), which may have increased injury in the foliar trial.

Non-target plant damage is an important consideration when using any herbicide (terrestrial or aquatic; Mudge and Haller 2010). Since it is common for homeowners, plant nurseries, and commercial farmers to utilize irrigation water from a nearby source for terrestrial plant species (Hodges and Haydu 2006), it is important to understand the effect of using water that has been recently treated with an aquatic herbicide. Although metsulfuron is not currently labeled for in-water (subsurface) use, it has shown activity as an in-water treatment at low concentrations against the aquatic plants giant salvinia (see Chapter 2), common duckweed

(*Lemna minor* L.), and Eurasian watermilfoil (*Myriophyllum spicatum* L.; EPA 2019a, b). Metsulfuron also degrades relatively quickly in water, especially under lower pH conditions (Grey and McCullough 2012), and exhibits a low toxicity profile to animals and aquatic organisms (Shaner 2014). Due to these desirable characteristics, it is a viable candidate for a full FIFRA Section 3 aquatic label. Currently, metsulfuron aquatic use is granted under a SLN label in Texas and can only be applied by federal and state agencies to control giant salvinia in public waters (i.e., freshwater sloughs, marshes, lakes, and other quiescent systems; Alligare 2019). Under this label, treated water from the application area may not be used for irrigation purposes and herbicide cannot be applied within 402 meters of any functioning potable water intake (Alligare 2019). This research will assist in determining irrigation restrictions for metsulfuron if a Section 3 aquatic label is obtained in the future.

3.4. Sources of Materials

[1] Bracy's Nursery, LLC., 64624 Dummyline Rd. Amite City, LA 70422.

[2] Osmocote®, The Scotts Company, PO Box 606 Marysville, OH 43040.

[3] Aquatic Plants of Florida, Inc., 8120 Blaikie Ct. Sarasota, FL. 34240.

[4] Timberline Top Soil, Oldcastle® Lawn & Garden, Inc., 900 Ashwood Pkwy. Atlanta, GA 30338.

[5] PRO MSM 60®, Alligare, LLC, 13 N. 8th Street Opelika, AL 36801.

[6] TeeJet®, Spraying Systems Co., P.O. Box 7900 Wheaton, IL 60187.

[7] Surf-AC® 910, Drexel Chemical Company, P.O. Box 13327 Memphis, TN 38113.

[8] SAS® software version 9.4, SAS Institute Inc., 100 SAS Campus Drive Cary, NC 27513.

Chapter 4. Summary and Conclusion

A series of outdoor mesocosm experiments were conducted in 2018 and 2019 at the Louisiana State University (LSU) Aquaculture Research Facility in Baton Rouge, LA to investigate the activity of metsulfuron-methyl against giant salvinia (*Salvinia molesta* D.S. Mitchell) as well as determine the impacts on non-target aquatic and terrestrial vegetation. Foliar rate titration trials were conducted to determine the most efficacious foliar rates of metsulfuron to control giant salvinia. Metsulfuron applied to the foliage of giant salvinia resulted in stunted plant growth and necrosis by 1 week after treatment (WAT) for all application rates evaluated. At 2 WAT, plants treated with 2.6 and 5.3 g active ingredient (a.i.) ha^{-1} began to recover by the production of new juvenile buds. Plants treated with 10.5 to 168.2 g a.i. ha^{-1} began to lose buoyancy and desiccate by 3 WAT. The majority of the plants treated with these rates of metsulfuron fell below the surface of the water by 6 WAT and by the conclusion of the trial (8 WAT), only a few necrotic, desiccated fronds remained at the surface of the water, with no observable regrowth. The calculated LD_{90} (lethal dose required to control 90% of the test population) of metsulfuron when applied to the foliage of giant salvinia was 3.83 g a.i. ha^{-1}. Despite plant recovery at a rate this low, it is evident how highly active metsulfuron is against giant salvinia when applied as a foliar treatment.

Mesocosm trials were conducted to determine if foliar applications of metsulfuron plus previously registered aquatic herbicides are compatible and if speed of injury and efficacy can be increased when applied to the foliage of plants. All treatments involving metsulfuron plus carfentrazone, diquat, flumioxazin, or glyphosate resulted in $\geq$ 30% plant injury 1 to 3 DAT, while metsulfuron alone provided 10% injury at 3 DAT. By 2 WAT, differences among metsulfuron treatments (alone and combination) with regard to the type and severity of plant injury were insignificant. All plants were necrotic and began to desiccate at 4 WAT, and the majority of the plants fell to the bottom of the container by the conclusion of the trial. At 8 WAT, all herbicide treatments resulted in ≥ 98% control. This research provides evidence that metsulfuron is compatible with carfentrazone, diquat, flumioxazin, or glyphosate when tank-mixed and applied to the foliage of giant salvinia. While plant control was not increased by the herbicide combinations, speed of injury and control were increased. Since metsulfuron requires several days to provide injury symptoms, the addition of these herbicides will not only provide faster control, but offer rapid visual markers (hours to 1 day), which can aid in distinguishing treated vs. untreated sites.

Additional metsulfuron titration trials were conducted to determine if subsurface applications of metsulfuron have activity on giant salvinia under static exposures. Plants exposed to metsulfuron at ≤ 5 μg a.i. L^{-1} exhibited visual injury by 2 WAT and became increasingly necrotic from 2 to 3 WAT; however, at 4 WAT, new buds began to form. By the conclusion of the trial, the majority of the herbicide injured fronds/plants exposed to these concentrations sunk to the bottom of the containers and the plants were healthy, actively growing, and similar in appearance to the control plants. Visual injury for plants exposed to metsulfuron at 10 to 80 μg a.i. L^{-1} was significant (> 50%) at 2 WAT. Unlike the lower concentration treatments, no plant recovery occurred at doses ≥ 10 μg a.i. L^{-1}. Plants were initially necrotic, began to lose buoyancy at 4 WAT, and continued to decline in health during the remainder of the trials. At the conclusion of the trials (10 WAT), > 98% of the plant material treated with doses ≥ 10 μg a.i. L^{-1} fell below the water surface with only a few desiccated, unhealthy fronds remaining at the surface of the water. The calculated EC_{90} (effective concentration required to control 90% of the test population) for metsulfuron applied subsurface against giant salvinia was 1.87 µg a.i. L^{-1}, which supports the notion that metsulfuron is highly active as an in-water treatment against giant salvinia.

Outdoor mesocosm experiments were also conducted to determine the sensitivity of non-target aquatic species to foliar applied metsulfuron, and to evaluate the phytotoxic effects of metsulfuron treated irrigation water on non-target aquatic and terrestrial species. Giant blue irises (*Iris giganticaerulea* Small.) were highly sensitive to all foliar application rates of metsulfuron evaluated (10.5 to 84.1 g a.i. ha^{-1}). At 2 WAT, plant growth was stunted and plants were chlorotic by 3 WAT. Metsulfuron treated plants became increasingly necrotic throughout the duration of the trial. At 7 WAT, plant dry weight was reduced 97 to 99%, regardless of herbicide rate, with no observable recovery. Herbicide symptomology occurred quickly for broadleaf arrowhead (*Sagittaria latifolia* Willd.) treated with metsulfuron. Plants exhibited stunted growth and necrosis by 3 DAT, with total plant death occurring by 2 WAT. At 3 WAT, yellow water lily (*Nymphaea mexicana* Zucc.) leaves that had come in direct contact with the herbicide spray solution were desiccated and plant injury increased as the herbicide continued to translocate through the plants. At 7 WAT, no regrowth had occurred and plants were reduced 84 to 87% in dry weight compared to the control. Broadleaf cattail (*Typha latifolia* L.) plants treated with metsulfuron at 10.5 and 21.1 g a.i. ha^{-1} continued to increase in plant height and biomass throughout the experiment, but plant growth and development was slower than the non-treated control. Plants treated with 42.1 and 84.1 g a.i. ha^{-1} displayed stunted growth and necrosis by 2 WAT and became increasingly necrotic throughout the remainder of the trials with no regrowth. At 7 WAT, there were no significant differences in dry weight biomass between the control and the metsulfuron treatments of 10.5 and 21.1 g a.i. ha-1, whereas the 42.1 and 84.1 g a.i. ha^{-1} treatments reduced plant dry weight by 85 and 91%, respectively. This research indicates that giant blue iris, broadleaf arrowhead, and yellow water lily are highly sensitive to foliar applications of metsulfuron at 10.5 to 84.1 g a.i. ha^{-1}. Although broadleaf cattail was not impacted by foliar application rates ≤ 21.1 g a.i. ha^{-1}, it was highly sensitive to rates of 42.1 and 84.1 g a.i. ha^{-1}.

Giant blue iris plants were not affected by metsulfuron applied in irrigation water at 1, 2.5, 5, 10, and 20 µg a.i. L^{-1}. However, plants treated with metsulfuron at 40 µg a.i. L^{-1} exhibited slight discoloration, reduced flowering and biomass was reduced 29% at 7 WAT. Metsulfuron applied in irrigation water at concentrations ranging from 1 to 40 µg a.i. L^{-1} had no impact cherry tomato (*Solanum lycopersicum* L. 'Sun Gold'), vinca [*Catharanthus roseus* (L.) G. Don. 'Pacifica Cherry Halo'], or soybean [*Glycine max* (L.) Merr. 'Asgrow® 5535'] plants.

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Vita

Will Prevost, a native of Raymond, Mississippi, enrolled in Mississippi State University in 2013 and earned a Bachelor of Science degree in agriculture engineering, technology, and business with a concentration in precision agriculture in December of 2017. After faced with the choice to either look for a job or pursue further education, he decided to return to school to obtain a Master of Science in agronomy specializing in aquatic weed management. After graduation with a M.S. from Louisiana State University, he will pursue a career in the field of weed science.