ABSTRACT

HOWELL, ANDREW WESLEY. Evaluations of Florpyrauxifen-benzyl and Unoccupied Aerial Systems to Manage Invasive Aquatic Plants in North Carolina and New Zealand. (Under the direction of Dr. Robert J. Richardson and Dr. Ramon G. Leon).

Invasive aquatic plants threaten freshwaters and associated environs worldwide. Resource managers frequently seek new control tactics to combat invasive plants, especially since registered aquatic herbicides remains limited. The development of the synthetic auxin herbicide, florpyrauxifen-benzyl, in the United States provides managers with another herbicide for invasive plant control.

Studies were conducted in New Zealand to evaluate florpyrauxifen-benzyl to control invasive submersed and emergent aquatic plants. In the first study, the submersed plants, [*Ceratophyllum demersum* L.], [*Lagarosiphon major* (Ridley) Moss], and [*Egeria densa* Planch.] were exposed to florpyrauxifen-benzyl at 30 and 50 µg a.i. L^{-1} in mesocosm settings for 8 weeks. Results indicated florpyrauxifen-benzyl at 30 µg L^{-1} provided CC_{80} (80% control compared to nontreated plant) of *L. major* and *E. densa* at 4 and 7 weeks after treatment (WAT), respectively. Neither rate provided control of *C. demersum*. In the second study, the emergent aquatic plants, [*Myriophyllum aquaticum* (Vell.) Verdc.] and [*Alternanthera philoxeroides* (Mart.) Griseb.] were exposed to foliar florpyrauxifen-benzyl applications. Greenhouse and outdoor mesocosm trials indicated single foliar applications of florpyrauxifen-benzyl provided >90% *M. aquaticum* control 4 and 8 WAT at rates ≥ 29.4 g ha^{-1}. *Alternanthera philoxeroides* was less sensitive than *M. aquaticum*, and repeat applications were required to achieve >94% control 12 WAT. The third experiment evaluated native and invasive submersed plant response to florpyrauxifen-benzyl under 21-day growth chamber conditions. Results indicated the New Zealand native plant, [*Myriophyllum triphyllum* Orchard], was highly sensitive to florpyrauxifen-benzyl, having

a dry weight 50% effective concentration (EC_{50}) value of 1.2 µg L^{-1}. The invasive species *L. major* and [*Elodea canadensis* Michx.] were less sensitive, with dry weight EC_{50} values of 35.4 and >107.86 µg L^{-1}, respectively. *Egeria densa* was most tolerant, as EC_{50} values were not achieved. Large-scale screening is needed to confirm florpyrauxifen-benzyl plant control in field sites.

Unoccupied aerial systems (UAS) have gained popularity for weed management. Further, some UAS provide opportunities to deliver herbicide applications. Unoccupied aerial application systems (UAAS) have successfully applied herbicide to terrestrial plants. However, studies describing UAAS sprayers in aquatic environments remains limited.

Studies in New Zealand and North Carolina investigated UAS and UAAS for aquatic plant mapping and management. In the first fourth study, an UAS was deployed to map and provide biomass estimates of Manchurian Wild Rice (MWR) [*Zizania latifolia* (Griseb.) Hance ex F. Muell.], an invasive semi-aquatic graminoid in New Zealand. Biomass estimates showed strong agreement with MWR plant height collected *in situ* ($r^2_{adj} = 0.61$) and the regression equation showed MWR accounted for 985.79 kg dry mass within the 13.61 ha study site. In the fifth study, herbicides were evaluated in field at low-carrier volumes (140 L ha^{-1}) to control [*Salvinia molesta* (D. S. Mitchell)] in North Carolina. Results indicated diquat (3136 g a.i. ha^{-1}) alone, glyphosate (4539 g a.e. ha^{-1}) alone, and metsulfuron-methyl (42 g a.i. ha^{-1}) alone achieved 86 to 94% visual plant control at 8 WAT. Carfentrazone (67 g a.i. ha^{-1}) ground based applications provided greater control than UAAS at 2 and 3 WAT, though neither application method was efficacious 8 WAT. Greenhouse results indicated diquat at ultralow carrier volumes (23 L ha^{-1}) reduced plant biomass ≥ 98% at 4 WAT. In the final studies, an UAAS delivered florpyrauxifen-benzyl as either foliar or directed in-water spray applications. Results from the

first case study showed [*Nymphoides peltata* (S.G. Gmel.) Kuntze] was visually controlled 80 to 99% by 6 WAT using foliar techniques. The second case study demonstrated UAAS directed in-water herbicide applications visually controlled [*Myriophyllum heterophyllum* Michx.] by 94% at 5 WAT. These data suggest UAAS provide an effective and efficient treatment option for floating-leaved and submersed plant management.

Evaluations of Florpyrauxifen-benzyl and Unoccupied Aerial Systems to Manage Invasive Aquatic Plants in North Carolina and New Zealand

by
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DEDICATION

"A true conservationist is a man who knows that the world is not given by his fathers, but borrowed from his children." – John James Audubon. This dissertation is dedicated to all those who devote their lives defending our natural lands, waters, and wildlife.

BIOGRAPHY

Andrew W. Howell grew up in Lee County, North Carolina exploring the local woods and waters in search of adventure. Andrew's love for the outdoors led him to his studies at North Carolina State University where he gained interest in weeds and their associated impacts on the landscape. Following his undergraduate degree in Crop Production, Andrew pursued a MS in Crop Science under the direction of Dr. Rob Richardson assessing novel boat-based sensing technologies to quantify submersed invasive plants. Upon completion of his MS, he was offered a PhD position under the direction of Dr. Rob Richardson and Dr. Ramon Leon evaluating the integration of unoccupied aerial systems and a newly registered aquatic herbicide to combat invasive aquatic plants. His PhD studies ultimately led Andrew to conduct research in New Zealand where he lived for six months. Andrew has remained active in numerous professional societies and conservation organizations throughout his educational career. He also founded the North Carolina State University collegiate chapter of Backcountry, Hunters, and Anglers, a North American organization that seeks to be the voice of our wild public lands, waters, and wildlife. Andrew plans to continue invasive species research and strives to provide technical advances for aquatic plant managers. In his free time, Andrew can be found in the great outdoors trout fishing, bowhunting, and backpacking.

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Significance of Aquatic Plant Management

The introduction of invasive aquatic plants (AIP) into freshwater systems frequently displaces native flora and fauna through resource competition resulting from the establishment of dense monotypic stands that limit light, carbon, and nutrient availability (Hofstra and Champion 2007; Wells et al. 1997; True-Meadows et al. 2016; Hussner et al. 2017). Similarly, AIP that produce high biomass yielding canopies (e.g., Hydrocharitaceae) regularly obstruct navigation, clog waterways used for irrigation and hydroelectric generation, and impede recreational opportunities (Carpenter and Lodge 1986; Clayton and Champion 2006; Langland 1996). Further, some AIP species promote vector-borne disease habitat (Orr and Resh 1999) and in some instances, host epiphytic cyanobacteria that can cause terminal illness of water birds (Wilde et al. 2005). Therefore, the conservancy of native aquatic plants is crucial for maintaining biodiversity within waterways (Madsen and Sand-Jensen 1991; Kovalenko et al. 2010; Hofstra et al. 2021), as native plant species are responsible for innumerable ecosystem services (Carpenter and Lodge 1986; Cyr and Downing 1988; Madsen et al. 2001; Petr 2000; Valley et al. 2004).

While AIP often threaten ecosystem processes, the monetary hurdles associated with these species are equally noteworthy. Studies have estimated that within the United States alone, more than $100 million dollars are allocated annually for AIP management (Rockwell 2003). These expenses include all tactics associated with AIP detection, monitoring, and control efforts. To date, the most effective method to mitigate the effects of AIP is through early detection and rapid response measures (EDRR) (Westbrooks 2004), as early detection minimizes the financial and environmental risks associated with plant invasions (Rejmànek and Pitcairn 2002; Hestir et al. 2008; Thum and Lennon 2009; Lambert et al. 2010). However, when AIP incursions do occur, aquatic resource managers must integrate active management tactics to combat the deleterious effects associated with AIP.

Biological, physical, and mechanical control options are available to control AIP; however, herbicides are generally the most economic, effective, and selective management tool used to control AIP (Hussner et al. 2017; Muller et al. 2021). When considering herbicide for management, it is imperative to acknowledge that applying herbicides for AIP management requires several prerequisites in order to be considered. Often, this includes regulatory and economic constraints, herbicide efficacy and selectivity, and the public's support of the herbicide treatment (Champion et al. 2002; Clayton 1996; Madsen 2000; Stallings et al. 2015). These factors directly influence the use of herbicides for restoring native plant populations within invaded waterways (Getsinger et al. 2008; Weber et al. 2020). When considering herbicide-based management approaches, practitioners must also consider target and non-target plant responses to the herbicide treatment, as AIP can be found in mixed assemblages with native plant species. Ultimately, this ensures appropriate recommendations for management action, especially since most available aquatic herbicides do not provide uniform control of AIP.

In the United States, there are currently sixteen herbicides available for aquatic weed management (Schardt and Netherland 2020). While many of the available herbicides provide reliable control of AIP (Enloe et al. 2022), some offer more favorable control characteristics than others do. A good example of the importance for proper herbicide selection concerns the AIP giant salvinia [*Salvinia molesta* (D.S. Mitchell)].

Giant salvinia is a free-floating aquatic fern that has progressively colonized wetland sites throughout the southern tier of the United States for the past 30 years (Johnson 1995; EDDMapS 2022). Once established, giant salvinia is difficult to control due to its productive growth (Kaufman and Kaufman 2007), and effective dispersal mechanisms (Nelson et al. 2001; Glomski and Mudge 2013). Herbicide applications targeting giant salvinia are often challenging due to limited accessibility and environmental constraints (e.g., cypress trees provided sanctuary). Subsurface (i.e., in-water) applications of the aquatic herbicides endothall dipotassium salt, fluridone, penoxsulam, and bispyribac-sodium have shown potential in reducing floating plant biomass under greenhouse and field settings (Glomski et al. 2003; Mudge et al. 2012; Glomski and Mudge 2013; Mudge and Netherland 2020). However, subsurface herbicide treatments can be cost-prohibitive or restricted due to local limitations (e.g., irrigation restrictions) which limits operational implementation. Therefore, foliar-applied herbicides, like glyphosate, have generally remained the most common treatment option for giant salvinia control. The contact herbicides diquat, flumioxazin, and carfentrazone-ethyl have also provided favorable control (Nelson et al. 2001; Glomski and Getsinger 2006; Richardson et al. 2008), and have been deployed as individual treatments or as tank mix partners. While several foliar applied herbicides have been successfully utilized to combat AIP like giant salvinia (Nelson et al. 2007; Richardson et al. 2008; Glomski and Mudge 2013; Mudge et al. 2016; Sartain and Mudge 2019; Prevost et al. 2021), there remains an interest to expand the current aquatic herbicide portfolio to improve management opportunity.

Managing Invasive Aquatic Plants in New Zealand

Although New Zealand is an island country, it has not remained exempt from AIP incursions. In New Zealand, AIP recurrently threaten freshwater ecosystems, posing considerable ecologic, economic, and recreational risk. The diverse aquatic environs endemic to New Zealand requires aquatic plant managers to regularly seek control tactics to combat AIP intrusions to minimize associated AIP hindrances. To date, dozens of AIP have been documented in New Zealand following introduction through the nursery and aquarium trades (Champion et al. 2002). The submersed plants, coontail [*Ceratophyllum demersum* L.], South African oxygen weed [*Lagarosiphon major* (Ridley) Moss], and Brazilian waterweed [*Egeria densa* Planch.] remain the most challenging to manage due to invasion potential and dense growth forms (Clayton and Champion 2006; Hofstra et al. 2018). Similarly, the marginal sprawling species, alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.] and parrotfeather [*Myriophyllum aquaticum* (Vell.) Verdc.], pose management challenges due to the formation of thick monospecific floating mats and the limited control options available (Hofstra and Champion 2010). Fortunately, free-floating (e.g., giant salvinia) and floating-leaved (e.g., water hyacinth [*Eichhornia crassipes* (Mart.) Solms]) AIP only account for a small portion of control efforts in the country.

New Zealand aquatic plant managers regularly pursue effective control options to eradicate or manage AIP to aid in recovery of desirable native habitats. Biological control agents, like grass carp (*Ctenopharyngodon idella*) and beetles (e.g., flea beetle [*Lysathia ludoviciana*]; alligatorweed beetle [*Agasicles hygrophila*]), have shown promise (Julien et al. 1995; Cilliers 1998; Garner et al. 2013) for controlling some AIP; however, the effectiveness of management relies heavily on localized infestation and environmental constraints (i.e., insect overwintering potential). Likewise, biological control agents are stringently governed and remain limited in areas where permitting restricts release (Clayton 1996; Hussner et al. 2017). Mechanical methods for emergent plant control do offer temporary reductions in plant biomass, but the effectiveness of mechanical techniques is often inadequate due to AIP propagative strategies (i.e., plant fragments from mechanical harvesters contributing to new plant populations). Therefore, the most frequently utilized management approach in New Zealand to control AIP is with herbicides (Hofstra and Champion 2010; Dugdale and Champion 2012).

In New Zealand, only two herbicides are labeled for submersed plant control, diquat-dibromide (photosystem I inhibitor) and endothall dipotassium salt (protein phosphatase inhibitor). The herbicides glyphosate (EPSP inhibitor), imazapyr (acetolactate synthase inhibitor), and metsulfuron-methyl (acetolactate synthase inhibitor) can be used to control emergent and floating AIP, but only under special use permitting from governing agencies. For perspective, the United States has 11 additional herbicides registered for aquatic site application (Gettys et al. 2020). A limited herbicide portfolio ultimately restricts management opportunity and promotes selection pressures that can select for herbicide-resistant AIP populations (e.g., fluridone resistant hydrilla [*Hydrilla verticillata* (L.f.) Royle] in Florida, USA; Michel et al. 2004), which further reduces future AIP control options (Richardson 2008). Therefore, there remains a clear need to evaluate additional herbicides as they become available to enhance current AIP management programs, while safeguarding native plant species like watermilfoil [*Myriophyllum triphyllum* Orchard], which may be frequently intermixed or adjacent to introduced AIP (Rattray et al. 1994). Evaluations of additional sites of action are presently required to enhance the stewardship of the currently labeled aquatic herbicides in New Zealand.

Florpyrauxifen-benzyl Herbicide

The herbicide florpyrauxifen-benzyl (synthetic auxin) was initially developed for weed management in cereal crops, namely rice production (Epp et al. 2016). In 2018, florpyrauxifen-benzyl was registered as two available formulations (emulsifiable and soluble concentrates) for aquatic site applications within United States. Synthetic auxin herbicides (e.g., 2,4-D) have remained a key management tool since development in the 1940's to control weeds in both cropping and noncropland landscapes (Peterson et al. 2016). Herbicides like florpyrauxifen-benzyl are distinct in both molecular function and mobility within plants since they mimic the endogenous plant-regulating hormone, indole-3-acetic acid. Auxin compounds are necessary for plant cell elongation and division, phototropic responses, apical dominance, and additional growth processes (Gaines 2020; Grossman 2010). Plants susceptible to synthetic auxin herbicides undergo rapid growth when transcription factor proteins responsible for plant regulation trigger uncontrolled gene expression (McCauley et al. 2020; Grossman 2010; Parry et al. 2009). This process ultimately initiates accumulation of abscisic acid, hydrogen peroxide, and ethylene, which leads to leaf senescence, cell death, and loss of plant turgor through multifaceted processes that remain under examination (McCauley et al. 2020; Grossman 2010). Synthetic auxin overload in susceptible plants typically show signs of apical epinasty, twisting, shattering, and curling of leaf and stem tissues.

Unlike non-selective contact herbicides like diquat-dibromide, synthetic auxin herbicides, like 2,4-D, triclopyr, and florpyrauxifen-benzyl generally behave as selective herbicides that do not typically adversely affect monocotyledons compared to dicotyledonous (broadleaf) plant species (Gettys et al. 2020; Madsen 2000). However, as part of the arylpicolinate class of auxin herbicides, florpyrauxifen-benzyl associates with binding site receptors (TIR1/AFB1-5) atypical of precursor synthetic auxin herbicides (e.g., 2,4-D belongs to the phenoxy-carboxylate class) (Hoyerova et al. 2018; Epp et al. 2016; Lee et al. 2014). While monocotyledons classically display lower sensitivity to synthetic auxin herbicides than dicotyledonous plants, previous research demonstrates florpyrauxifen-benzyl is effective for controlling several monocotyledon

AIP (e.g., Hydrocharitaceae) (Richardson et al. 2016; Haug et al. 2021; Sperry et al. 2021). Further, florpyrauxifen-benzyl has ~100 times lower use rate pattern than 2,4-D and triclopyr, and has been classified as a reduced risk herbicide by the United States Environmental Protection Agency (USEPA 2017).

In the United States, florpyrauxifen-benzyl has enhanced AIP management by controlling common invasive weeds like *H. verticillata*, *Myriophyllum* spp., and crested floatingheart [*Nymphoides cristata* (Roxb.) O. Ktze.] at low herbicide concentrations (e.g., <3-50 $\mu g\ L^{-1}$) (Netherland and Richardson 2016; Richardson et al. 2016; Beets and Netherland 2018; Sperry et al. 2021). However, it should be noted that florpyrauxifen-benzyl rapidly degrades through photolysis (1- to 2-d half-life), with secondary degradation occurring through hydrolysis as water pH increases (pH 7 to 9; 111- to 2-d half-life, respectively) (Heilman and Getsinger 2018; MDA 2018). Due to herbicide degradation pathways, managers must consider environmental constraints that could lead to reduced herbicide efficacy (e.g., high turbidity levels; high alkaline waters; algal presence). Nevertheless, a field application experiment evaluating florpyrauxifen-benzyl to selectively control dense dioecious *H. verticillata*, found that while the herbicide rapidly dissipated within 6-hr after application, initial herbicide activity provided near complete plant control of the plant for 133 d following application (Sperry et al. 2021).

A novel systemic herbicide like florpyrauxifen-benzyl that has rapid plant absorption (Haug et al. 2021) and a favorable toxicological profile (Buczek et al. 2020) could offer an additional tool aquatic plant managers deploy for selective AIP management strategies in New Zealand. However, literature describing florpyrauxifen-benzyl efficacy on common New Zealand AIP remains limited and non-target herbicide evaluations are needed to guide registration of the herbicide in the country.

Synopsis of Unoccupied Aerial Systems (UAS)

Over the past several decades, satellite and airborne optical sensors have aided in surveys for AIP delineation (Ackleson and Klemas 1987; Nelson et al. 2006; Hestir et al. 2008; Anderson et al. 2021). Combining remotely sensed datasets with computer-based programs and geographic information systems (GIS) (e.g., ArcGIS; GRASS GIS; ENVI; RStudio), has further enhanced mapping performance (Shaw 2005; Santos et al. 2016; Petrasova et al. 2017). Successful measurements of submersed and emergent AIP has been achieved using true-color and multispectral imagery (Ackleson and Klemas 1987; Peñuelas et al. 1997; Martin et al. 2010). However, the expenditures associated with repeated measurements over large spatial scales often limits the use of purchased satellite flights and airborne imaging missions (Underwood 2003). Multispectral open-source sensors, like Sentinel-2 and Landsat 7 TM/ETM+, do provide the necessary spectral ranges to assess broad AIP dynamics, but these platforms typically lack the spatiotemporal resolution (e.g. pixel size of 10 to 30 m^2 and ~5 to 15 days, respectively) required to evaluate localized AIP distributions or differentiate amongst alike aquatic plant species.

With the advent of consumer available unoccupied aerial systems (UAS), aquatic plant managers now have the opportunity to assess AIP community dynamics at very high spatial resolution (pixel sizes ~1 to 3 cm^2) at user defined temporal resolutions (min to hrs). Further, UAS cost a fraction of a single multispectral image from a commercial airborne or satellite sensing platform (Baluja et al. 2012; Turner et al. 2012; Candiago et al. 2015). Still, limitations exist with true-color or multispectral UAS platforms when surveying aquatic vegetation, specifically submersed aquatic vegetation (SAV). Plant physiological and environmental factors (e.g. turbidity; fetch; sun angle geometry; weather) often creates impediments for evaluating SAV above the water column. Nevertheless, successfully SAV detection and quantification has occurred in lentic and lotic systems deploying UAS (Chabot et al. 2016; Brooks et al. 2019; Kislik et al. 2020).

To date, several UAS studies have evaluated the performance of true-color sensors to quantify AIP within waterways, support EDRR efforts, and gauge success of active management (e.g., Chabot et al. 2016; Hill et al. 2017; Brinkhoff et al. 2018; Jochems et al. 2021). Although true-color imagery does provide high spatial resolution data, the spectral and radiometric range can limit the accuracy of repeated measurements. Like true-color sensors, multi- and hyperspectral sensor mounted to UAS have successfully mapped AIP in complex aquatic systems to overcome spectral and radiometric restrains. For example, Samiappan et al. (2017) utilized a multispectral unit fitted on UAS to provide mapping classification of the invasive graminoid, *Phragmites australis*, which is difficult to map via ground based methods due to thick monoculture stands. Similarly, other researchers have effectively deployed multispectral sensors on UAS for spatiotemporal monitoring of AIP herbicide treatments (Sartain et al. 2019; Benjamin et al. 2021). Nevertheless, the ease of application and low costs associated with true-color UAS imagery have not made multi- and hyperspectral sensors commonplace for AIP monitoring.

In addition to providing a platform for small optical imagers and other sensing devices for AIP monitoring, UAS provide opportunities to remotely deliver herbicide to plant targets (Göktoğan et al. 2009; Milling 2018; Hunter et al. 2020; Rodriguez et al. 2022). Recently, UAS sprayers (unoccupied aerial application systems) have gained recognition among herbicide application sites which restrict ground-based spray operations (Xue et al. 2016; Lan et al. 2017; Wang et al. 2019). The benefits of UAS sprayers include improved safety of the herbicide application (i.e., reduced herbicide exposure), and the lack of on-board crew reduces inherent risks associated with traditional aerial application techniques (Sheets 2018; Vu et al. 2019). Likewise, UAS sprayers can traverse tight application sites (e.g., ≤ 4 ha; tree obstructions) that would be expensive or prohibited to apply with plane or helicopter (He 2018; Otto et al. 2018). Further, UAS sprayers allow for site- and species-specific weed management strategies (Hunter et al. 2020; Ahmad et al. 2020; Martin et al. 2020; Pathak et al. 2020).

While integration of UAS sprayers has largely occurred in terrestrial weed management scenarios, the opportunity exists to implement these spray systems in aquatics. For example, an autonomous UAS sprayer was used simulate the application of herbicide using dye to demonstrate a proof-of-concept model for managing *A. philoxeroides* and *S. molesta* in Australia (Göktoğan et al. 2009). However, no literature currently describes UAS sprayers, suggested herbicides, or UAS spray strategies for aquatic weed management in the United States. Nevertheless, aquatic plant managers have expressed interest to integrate UAS sprayers to overcome environmental hindrances (e.g., lack of boat launch facilities) regularly experienced when conducting aquatic AIP management. To date, there remains a need to evaluate the performance of UAS sprayers and select herbicides delivered from these systems to guide future AIP management strategies.

In summary, many UAS platforms are available for AIP monitoring and plant control, and managers should carefully consider which systems best serve the purpose of the intended survey or herbicide application. Likewise, managers must adhere to the necessary regulatory confines (i.e., FAA Part 107) that might limit immediate operation. For example, in regions were visual line of sight is not possible to achieve the flight mission, UAS take-off and landing will likely require the use of watercraft in order to maintain visual line of sight. For UAS sprayers, managers must not only consider the weight of the unit payload (e.g., sprayer or spreader attachment ≤ 24.9 kg), but the overall application constraints of the system (e.g., tank size; manual vs. autonomous operations). Similarly, mangers desiring to apply herbicides with UAS sprayer platforms should carefully review all regulatory and licensing requirements before purchase and operation (i.e., conform to FAA Part 137: Agricultural Aircraft Operations, in addition to FAA Part 107). The application of UAS sprayer platforms and application techniques developed through research remains crucial in order to implement these novel application systems, and the utilization in aquatics remains under investigation.

Research Objectives

Chapter 2: The objective of this study was to demonstrate florpyrauxifen-benzyl for potential operational use in New Zealand to control coontail [*Ceratophyllum demersum* L.], South African oxygen weed [*Lagarosiphon major* (Ridley) Moss], and Brazilian waterweed [*Egeria densa* Planch.], all which have high biosecurity concern with limited herbicides available for management.

Chapter 3: The objective of this research was to evaluate foliar application rates of florpyrauxifen-benzyl that provide effective control of the marginal invasive aquatic plants, parrotfeather [*Myriophyllum aquaticum* (Vell.) Verdc.] and alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.], for water resource management in the United States and New Zealand.

Chapter 4: The objective of this study was to implement small-scale screening analyses of florpyrauxifen-benzyl to evaluate the relative sensitives of native and invasive submersed plant species commonly found in New Zealand using dose–response protocols.

Chapter 5: The objective of this pilot project was to provide a timely and systematic appraisal of invasive Manchurian wild rice [*Zizania latifolia* (Griseb.) Hance ex F. Muell.] standing biomass using unoccupied aerial system imagery and photogrammetry modeling to assess plant growth, population dynamics, and delineate post-treatment herbicide responses in the Wellington Region, New Zealand.

Chapter 6: The objectives of this research were to (1) evaluate the efficacy of aquatic and experimental herbicides at reduced carrier volumes for giant salvinia [*Salvinia molesta* (D. S. Mitchell)] eradication efforts in North Carolina, (2) document the performance of carfentrazone herbicide deployed from an unoccupied aerial application system to control giant salvinia, and (3) determine if ultralow carrier volumes provide effective control of giant salvinia using the herbicides diquat, flumioxazin, and glyphosate.

Chapter 7: The objectives of these case studies were to (1) determine the feasibility of unoccupied aerial application systems for foliar and directed in-water application strategies with florpyrauxifen-benzyl herbicide, (2) evaluate if unoccupied aerial application systems provide an effective application strategy for yellow floatingheart [*Nymphoides peltata* (S.G. Gmel.) Kuntze] and variable-leaf watermilfoil [*Myriophyllum heterophyllum* Michx.] control, and (3) identify the application constraints that currently exist with unoccupied aerial application system technology in aquatics.

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CHAPTER 2: Evaluation of florpyrauxifen-benzyl to control three problematic submersed macrophytes in New Zealand

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Introduction

Exotic aquatic plant invasions continually threaten New Zealand's freshwaters, posing substantial ecologic, economic, and recreational risk. Of particular concern are invasive submersed macrophytes, which commonly develop as high biomass yielding monocultures (Wells et al. 1997; Hofstra et al. 2018). Once established, these aggressive submersed weeds have the potential to obstruct waterways, which can adversely affect water supply, power generation, and ecosystem services (Carpenter and Lodge 1986; Clayton 1996). The unique and diverse aquatic environments endemic to New Zealand require water resource managers to regularly deploy control tactics to combat submersed plant incursions.

Dozens of exotic macrophytes are documented in New Zealand (Champion et al. 2002, MPI 2020) though the submersed plants, *Ceratophyllum demersum* L. (dicot), *Lagarosiphon major* (Ridley) Moss (monocot), and *Egeria densa* Planch. (monocot) remain the most historically problematic due to broad-invasion potential and dense growth form habits (Clayton and Champion 2006; Hofstra et al. 2018). Among invaded systems, populations of *C. demersum*, *L. major*, and *E. densa* are regularly monitored (e.g., LakeSPI; Clayton and Edwards 2006) and targeted for control using combinations of physical, biological, and chemical management approaches (Hussner et al. 2017). Mechanical control techniques can be successful when plant fragmentation and regeneration is of minimal concern (Clayton 1996; Redekop et al. 2016). Likewise, grass carp (*Ctenopharyngodon idella*) have been effective for invasive plant control (Champion 2018), but are non-selective grazers, which may cause concern for native submersed plant foraging. Additionally, the effectiveness of grass carp is constrained by the waterbody characteristics (e.g., the ability to contain the grass carp) (Hofstra et al. 2014). For targeted removal programs, large-scale plant suppression or eradication often succeeds when chemical control is deployed due to invasive plant morphology and costs associated with problematic species (Howard-Williams et al. 1987; Bickel 2012; de Winton et al. 2013). New Zealand currently has just two herbicides registered for aquatic site applications, diquat dibromide (Photosystem I inhibitor) and endothall dipotassium salt (Protein phosphatase inhibitor). While both herbicides readily control *C. demersum* and *L. major* (Wells and Champion 2010; Wells et al. 2014), only diquat has shown effective control of *E. densa* (Hofstra and Clayton 2001; Skogerboe et al. 2006).

A limited aquatic herbicide portfolio narrows management opportunity and increases selection pressure among target plant populations. Richardson (2008) noted the implications of resistance management in aquatic weed control scenarios and how repetitive use patterns, or herbicide modes of action (MOA), can select for resistant populations among invasive plant biotypes (e.g., fluridone resistant *Hydrilla verticillata* (L.f.) Royle in Florida, USA; Michel et al. 2004). Other functional constraints, like potential irrigation and swimming restrictions or selectivity to native plants following herbicide application, can further limit or eliminate management opportunities when only two primary herbicides may be considered (Champion et al. 2019). Thus, there remains a need to evaluate additional MOA to expand management options for invasive aquatic plants and enhance stewardship of the currently herbicides in New Zealand.

The recent development of a unique auxin-mimic compound, florpyrauxifen-benzyl (arylpicolineate subclass), in the United States provides water resource managers with an additional herbicide. Florpyrauxifen-benzyl has been classified as a reduced risk herbicide by the United States Environmental Protection Agency (U.S. EPA), and is registered in aquatic systems to target invasive weed species like *H. verticillata* and *Myriophyllum spicatum* L. (US EPA 2017). Additionally, florpyrauxifen-benzyl is suspected to degrade primarily via aqueous photolysis under aquatic field dissipation scenarios (Meléndez et al. 2017). While monocots typically exhibit lower sensitivity than dicots to auxin-mimic herbicides (e.g., 2, 4-D and triclopyr), previous research demonstrated potential use for controlling several invasive macrophyte species including problematic submersed monocots (Richardson et al. 2016; Haug 2018). Literature also reports florpyrauxifen-benzyl effectively reduces total biomass of several Hydrocharitaceae and *Myriophyllum* spp. at lower use rates (e.g., <3-50 µg L^{-1}) than the existing herbicides available in New Zealand (Netherland and Richardson 2016; Richardson et al. 2016). A novel systemic herbicide chemistry with rapid plant absorption (Haug et al. 2021), like florpyrauxifen-benzyl, could provide an additional tool that water resource managers deploy to combat invasive submersed species in New Zealand. However, there are no published control data on the problem species *C. demersum, L. major,* or *E. densa* using florpyrauxifen-benzyl.

The objective of this study was to demonstrate an additional MOA for potential operational rates of florpyrauxifen-benzyl (30 and 50 µg L^{-1}) to control *C. demersum, L. major*, and *E. densa*, which have high biosecurity concern in New Zealand. Previous experiments suggest rapid herbicide activity and symptomology among sensitive plant species (e.g., *H.*

verticillata and *Myriophyllum spicatum L.*). Therefore, target herbicide concentrations selected for this study used previous florpyrauxifen-benzyl dose response metrics as reference for Hydrocharitaceae (i.e., *L. major* and *E. densa*) control in this study (Netherland and Richardson 2016; Richardson et al. 2016). This experiment occurred simultaneously with the product label registration of florpyrauxifen-benzyl (ProcellaCOR®, SePRO, Carmel, IN, USA) in the United States.

Materials and Methods

Experiments occurred at the National Institute of Water and Atmospheric Research (NIWA) Ruakura, North Island, New Zealand mesocosm facility. Plants were field collected from Lake Karāpiro (*C. demersum* and *E. densa*) and Lake Rotoiti (*L. major*), then cultured in outdoor tanks filled with dechlorinated water. Individual *C. demersum*, *L. major*, and *E. densa* plants were established using 10 cm apical shoots placed in 300 ml pots filled with topsoil (72% dry matter; 1.3 g phosphorus and 0.13 g nitrogen kg^{-1} dry weight soil) and covered with washed river sand (~1 cm). Respective pots (n = 14 pots of individual species per tank) were then placed in species specific 176 L treatment tanks to provide a minimum eight weeks of development prior to treatment. To minimize algae, help regulate water temperature, and reduce bleaching of surface biomass, 50% shade cloth was placed over each tank at establishment. Each tank remained at a consistent water level via addition of freshwater and contained one aeration stone (6-10 L min^{-1}) to suppress algae accumulation over the eight-week study period.

Plants were treated 16 January 2018 with a 300 g L^{-1} suspension concentrate formulation (SLF-9522) of florpyrauxifen-benzyl[1] to target 30 or 50 µg L^{-1} as in-water applications using a dilute solution of product, mixed with municipal-water as carrier, to achieve target treatment dose among each experimental unit. The 50 µg L^{-1} florpyrauxifen-benzyl treatment closely reflects the maximum active ingredient (a.i.) concentration rate for in-water applications in a single application in the United States. Herbicide treatments (30 or 50 µg L^{-1}) were applied independently among each species treatment tank and replicated six times. Six untreated control tanks were also included per macrophyte species. At treatment, one untreated experimental unit representing each species was pre-harvested to obtain baseline shoot and root biomass (g dry weight) to evaluate untreated plant growth over the evaluation period. Water temperature averaged 21.7° C and 8.3 pH across tanks at treatment.

Throughout the 8-week static exposure, observations of plant appearance (morphologic response to herbicide) and visual percent control estimates compared to untreated controls (0% = no control; 100% = complete necrosis) were recorded weekly. Among respective observation timepoints, photographs of each experimental unit were taken to serve as standards for visual percent control estimates during analysis. Shoot and root biomass from each tank was harvested following the completion of the study and dried at 60° C for 72 hrs to obtain constant dry mass (g dry weight). Only shoot biomass was collected for *C. demersum* since plants in the Ceratophyllaceae family lack true roots.

Due to rapid shoot terminal abscission (ca. 5 to 7 days after treatment) among *L. major* and *E. densa* to both florpyrauxifen-benzyl rates tested (30 or 50 µg L^{-1}), a nested experiment occurred to observe the potential regrowth of abscised shoots. Occurring 4 WAT (weeks after treatment), three 10 cm long apical shoots from each experimental unit (n = 18 shoots $treatment^{-1}$ $species^{-1}$) were manually cut and weighed (fresh weight), then placed in respective 11 L mesocosms filled with freshwater (N = 9 tanks; 3 tanks $species^{-1}$ at the three exposures tested: untreated control, 30 and 50 µg L^{-1} florpyrauxifen-benzyl) for viability monitoring. Mesocosms remained at a constant water level, covered with 50% shade cloth, and provided with aeration during the four-week evaluation period. Stem lengths (mean of n = 3 randomly selected stems) and visual observations of symptomology were noted at times zero, two, and four-week evaluations to examine potential plant fragment viability (growth or decline in plant structure) compared to the untreated controls. Shoot biomass and mean three-stem lengths were collected at the end of the observation period to gauge fragment viability or decline based on initial herbicide exposure to individual species response of simulated allofragmentation.

Data were analyzed using RStudio[2] 'base', 'agricolae', and 'dplyr' packages to provide statistical and visual representation of herbicide dose by species response, and to examine normality (R Core Team 2020; de Mendiburu 2020; Wickham et al. 2021). Plant dry weights were evaluated using ANOVA (analysis of variance) and separated using Fisher's Protected LSD at the $P < 0.05$ significance level. Visual control estimates were subjected to Student's t-test to separate treatment means at the $P < 0.05$ significance level. Local polynomial regression models where then utilized to calculate critical control values (CC_{80}: weeks after treatment to reach 80% visual control). The untreated control was not included for visual control analyses since it was a constant value of zero. *Ceratophyllum demersum* CC_{80} values are not reported since 80% visual control did not occur during the eight-week study duration.

Results and Discussion

Insert your main body text here. Both *E. densa* and *L. major* untreated control plants increased in biomass (μ = 2.64-4.96 times, respectively) compared with the pre-treatment harvest, suggesting conditions for the Hydrocharitaceae were favorable. *Lagarosiphon major* and *E. densa* displayed herbicide symptomology within 24 to 48 hours after treatment (HAT), with apical bending and node swelling appearing as first symptoms. *Ceratophyllum demersum* untreated control biomass did not increase over the study period (μ = 1.03 times) respective to the pre-treatment harvest.

Of the three species tested in the eight-week study, *L. major* had the greatest sensitivity to florpyrauxifen-benzyl (Table 1; Figure 1). Within 72 HAT, *L. major* proximal leaves were observed abscising from the stem, with regular shoot fragmentation occurring 5 to 7 days after treatment (DAT) among tanks treated with florpyrauxifen-benzyl 30 and 50 μg L^{-1} rates (Figure 2A). Within 9 DAT, all *L. major* treated plants experienced comparable leaf shattering and stem abscission, internode lengthening and some stem necrosis, with complete plant canopy collapse occurring within 2 WAT. In general, *L. major* abscised stem fragments floated at the water surface (prompted fragmentation viability investigation). Significant reduction to *L. major* shoot and root biomass occurred among plants treated with 30 μg L^{-1} florpyrauxifen-benzyl ($P < 0.05$), with complete necrosis of all plant material when exposed to 50 μg L^{-1} (Table 1). Within 3 WAT, 50 μg L^{-1} florpyrauxifen-benzyl achieved CC_{80} of *L. major*, with CC_{80} occurring among 30 μg L^{-1} treated plants 4 WAT. However, there was no difference between the 30 and 50 μg L^{-1} treatment rates for *L. major* shoot and root reduction (Student's t-test, $p = 0.364$ and 1, respectively). Following the 4 WAT evaluations, continued necrosis occurred among *L. major* plants, with almost no viable plant material discovered 8 WAT for plants treated at the low rate.

Upon initial observations, *E. densa* did not appear as sensitive to florpyrauxifen-benzyl as *L. major*, though terminal epinasty and internode twisting began within 3 to 5 DAT at both the low and high florpyrauxifen-benzyl rates (Figure 2B). Within 9 DAT, treated *E. densa* plants exhibited epinasty and increased internode length between both treatment rates, with visual observation of nodes turning bright orange/brown at the 50 μg L^{-1} rate. *Egeria densa* began showing signs of chlorosis and node expansion 2 WAT at both rates tested. At this time, stems became brittle and fragmentation was common when the water was agitated. Florpyrauxifen-benzyl did decrease *E. densa* shoot and root biomass (μ = 1.9 - 2.9 times that of the untreated control group) at both rates tested ($P < 0.05$; Table 1). While *E. densa* was not completely controlled 8 WAT, florpyrauxifen-benzyl 30 μg L^{-1} treatments did provide CC_{80} 7 WAT (Figure 1). Still, there was no difference among treatment rates in reducing *E. densa* shoot or root biomass (Student's t-test, $p = 0.417$ and 0.272, respectively). Variation in herbicide sensitivity between species belonging to the same family can be expected, and our findings are consistent with a previous small-scale study, which noted lower absorption and translocation potential of florpyrauxifen-benzyl for *E. densa* than another Hydrocharitaceae species, *H. verticillata* (Haug et al. 2017). An interesting phenomenon did occur among *E. densa* 50 μg L^{-1} florpyrauxifen-benzyl treatments, as two experimental units exhibited flowering 3 and 6 WAT. Flowering in these tanks may be linked to plant stress induced from the synthetic auxin characteristics of florpyrauxifen-benzyl, as plant growth regulators (i.e., auxin mimic herbicides) are involved in ethylene simulation and signaling pathways that control flower initiation (Bielach et al. 2017). A similar flower stimulation response was reported for the submersed species, *Myriophyllum sibiricum* Komarov, to the auxin mimic herbicide, 2-4,D, when exposed at a 10 μg L^{-1} rate (Forsyth et al. 2007). Although some treated *E. densa* biomass remained viable 8 WAT, a longer exposure may have resulted in greater observed control since much of the plant material collected at harvest showed signs of chlorosis and necrosis at the nodes (some stem fragmentation ensued when agitated).

While not as sensitive as the other species tested, *C. demersum* did show signs of chlorosis and terminal epinasty at the florpyrauxifen-benzyl 30 μg L^{-1} rate, with increased stem brittleness discovered at 50 μg L^{-1} rate 9 DAT. At the 2 WAT evaluations, all *C. demersum* tanks treated with florpyrauxifen-benzyl 50 µg L^{-1} experienced canopy collapse, with analogous plant structure decline beginning amongst the 30 µg L^{-1} plant treatments; this plant canopy response was not observed for the *C. demersum* untreated control tanks. Final collapse of the *C. demersum* plant canopies occurred 5 to 6 WAT for tanks treated with florpyrauxifen-benzyl at 30 µg L^{-1}.

Ceratophyllum demersum biomass was reduced at both florpyrauxifen-benzyl rates ($P < 0.05$, Table 1). Plant suppression (collapsed plant canopy) occurred simultaneously for both florpyrauxifen-benzyl 30 and 50 µg L^{-1} rates 4 WAT, although complete control did not occur 8 WAT (Table 1; Figure 1). While *C. demersum* plants treated with 50 µg L^{-1} florpyrauxifen-benzyl had greater biomass reduction than did the 30 µg L^{-1} rates, no significant decrease occurred between rates tested (Student's t-test, $p = 0.534$). Necrosis was not observed among any *C. demersum* tanks and CC_{80} was not achieved using either florpyrauxifen-benzyl rate tested.

Redekop et al. (2016) reported the dispersion potential of *E. densa* and *L. major* following simulated allofragmentation in an untreated flowing system and noted stem regeneration four weeks after fragmentation (N=11-50% not regenerated). To evaluate the potential viability of abscised (herbicide induced) apical shoots forming new plant populations from the treated plants in this study, we observed individual species response to simulated allofragmentation (10 cm long plant stems) over a four-week period (concurrent with 8-week tank study). All *C. demersum*, *E. densa*, and *L. major* fragments collected from tanks dosed with florpyrauxifen-benzyl were sunken at the zero, two, and four-week evaluations, while untreated fragments remained buoyant. Further, no growth (no increased shoot weight or length) occurred for any species tested at the two or four week evaluations at either florpyrauxifen-benzyl rate. For *L. major*, complete necrosis occurred (no observed biomass) among the 50 µg L^{-1} treatments at the 4-week evaluation, while the untreated control shoot length had doubled ($\mu = 2.03$ times) at the end of the observation period. Similarly, *E. densa* displayed no fragment viability compared to the untreated controls, having shoot length reductions (μ = 2 and 3 cm shorter, respectively) at the two and four-week evaluations (10 cm initial stem length). *Ceratophyllum demersum* stem fragments from the 30 and 50 µg L^{-1} displayed no increase or decrease in stem length at the end of the study period, however the average stem length within the untreated control group increased 40% (untreated control μ = 14 cm stem length). Likewise, *C. demersum* stems from treated tanks were brittle with twisted internodes at the four-week evaluation. Though observational, it is hypothesized induced allofragmentation following an application of florpyrauxifen-benzyl at 30 or 50 µg L^{-1} treatment rates would not likely increase stem fragment viability among the study species. However, operational situations would benefit from future studies comparing herbicide dose response with the potential regrowth of *C. demersum*, *E. densa*, and *L. major* following shoot dislocation of treated plants within hours, or days, after herbicide exposure to test fragment viability.

In conclusion, florpyrauxifen-benzyl has potential for managing these three problematic submersed macrophytes in New Zealand, particularly *L. major* which was the most sensitive species evaluated. Further, there was no difference between rates tested among any of the species studied (Table 1). Therefore, the low rate (30 µg L^{-1}) florpyrauxifen-benzyl showed effectiveness in controlling *L. major* and *E. densa*, providing another chemical control option for *E. densa* management other than the sole chemistry, diquat dibromide (Hofstra and Clayton 2001). While herbicide response at both tested rates did not provide complete control of *C. demersum*, florpyrauxifen-benzyl did reduce the plant canopy stature, which may be applicable when plant canopy suppression is the primary intention. For complete control of *C. demersum*, future research should evaluate combinations of available herbicides in New Zealand with application rates tested in this trial, and the influence of water chemistry on florpyrauxifen-benzyl efficacy (e.g., water pH of application site and degradation rates). Future research should also evaluate the operational rates tested in the study with likely sensitive native species (e.g., *Myriophyllum triphyllum* Orchard), and assess how concentration and exposure-times (CET) could influence use patterns for *C. demersum*, *L. major*, and *E. densa* management (e.g., Beets et al. 2019).

Sources of Materials

[1]ProcellaCOR SC, SePRO Corporation, Carmel, IN 46032.

[2]R version 4.0.3, R Foundation for Statistical Computing, Vienna, Austria.

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TABLES

Table 2-1. Shoot and root treatment response metrics for three problematic submersed macrophytes following an eight-week static exposure to florpyrauxifen-benzyl.

	Egeria densa		*Ceratophyllum demersum*	*Lagarosiphon major*	
Treatment	Shoot	Root	Shoot	Shoot	Root
			Dry Biomass[a,b]		
Untreated control	14.08 ± 2.61 (a)	1.87 ± 0.80 (a)	15.24 ± 2.95 (a)	27.38 ± 3.37 (a)	10.6 ± 5.48 (a)
florpyrauxifen-benzyl 30 μg L^{-1}	4.85 ± 1.91 (b)	0.41 ± 0.13 (b)	9.45 ± 0.58 (b)	3.10 ± 3.10 (b)	0.01 ± 0.01 (b)
florpyrauxifen-benzyl 50 μg L^{-1}	7.25 ± 1.00 (b)	0.74 ± 0.19 (ab)	7.90 ± 1.94 (b)	0.01 ± 0.01 (b)	0.01 ± 0.01 (b)
			% Reduction[c,d]		
florpyrauxifen-benzyl 30 μg L^{-1}	65.60 ± 13.47	78.10 ± 6.70	38.00 ± 3.78	88.70 ± 11.31	99.99 ± 0.07
florpyrauxifen-benzyl 50 μg L^{-1}	48.51 ± 7.10	60.60 ± 10.37	48.10 ± 12.70	100.00 ± 0.00	99.99 ± 0.07

[a]Biomass means (g dry weight ± SE) within columns with the same letter do not significantly differ according to Fisher's Protected LSD ($P < 0.05$).
[b]Due to *C. demersum* morphology, root biomass metrics are not provided.
[c]Percent reduction means (1 - treatment mean/untreated control mean) with ± SE depict biomass reduction compared to untreated control group.
[d]No significant difference detected between treatments relative to the untreated control group using Student's t-test ($P < 0.05$).

FIGURES

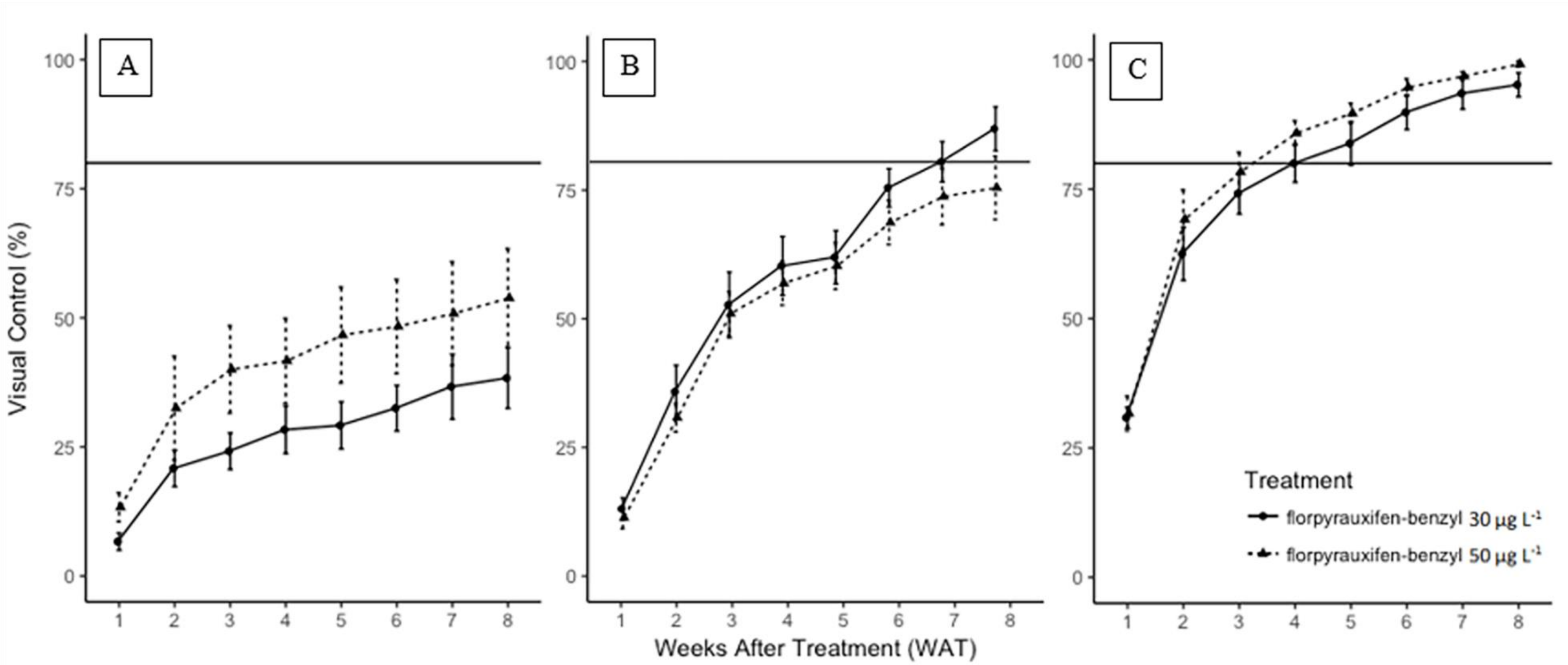

Figure 2-1. Week after treatment (WAT) visual control estimates of **A).** *C. demersum*, **B).** *E. densa*, and **C).** *L. major* treated with two rates of florpyrauxifen-benzyl. Visual control estimates expressed as a percentage control of treated plants compared to untreated control (0% = no control; 100% = complete necrosis). Discrete points with bars represent the mean ± SE; (n=6). Critical control values derived from local polynomial regressions are represented by the horizontal line set to CC_{80} (WAT to reach 80% control): *C. demersum* = NA, *E. densa* = (30 μg L^{-1}: 7WAT; 50 μg L^{-1}: NA), and *L. major* = (30 μg L^{-1}: 4WAT; 50 μg L^{-1}: 3WAT). No significant difference detected between treatments using Student's t-test (P < 0.05).

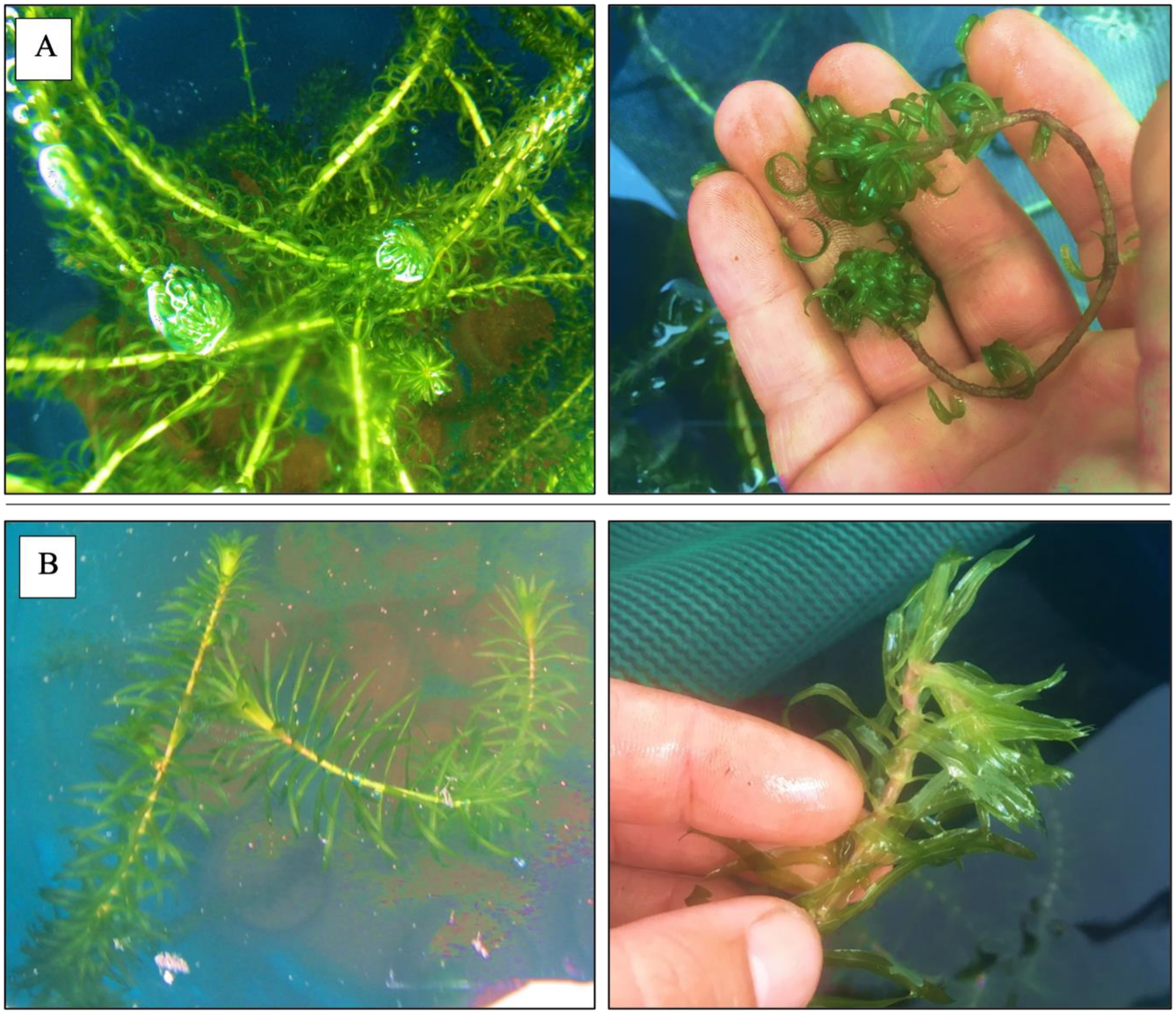

Figure 2-2. Leaf shattering response of *L. major* (A), and apical epinasty response of *E. densa* (B), ca. 7 DAT following static florpyrauxifen-benzyl 50 μg L^{-1} exposure (A and B; left: untreated control; right: treated tank).

CHAPTER 3: Response of parrotfeather watermilfoil and alligatorweed to foliar florpyrauxifen-benzyl applications

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Howell AW, Hofstra DE, Heilman MA, Richardson RJ (2022) Response of parrotfeather watermilfoil and alligatorweed to foliar florpyrauxifen-benzyl applications. J Aquat Plant Manage 60.

Abstract

The emergent aquatic plants, parrotfeather watermilfoil [*Myriophyllum aquaticum* (Vell.) Verdc.] and alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.] threaten native ecosystem services within invaded regions throughout the world. The registration of the auxin herbicide, florpyrauxifen-benzyl, in the United States provides water resource managers with another herbicide for weed control. Currently, available aquatic herbicides do not control these marginal weed species with consistency. Research was conducted in the United States (North Carolina) and New Zealand to evaluate foliar applications of florpyrauxifen-benzyl for parrotfeather watermilfoil and alligatorweed control. Greenhouse and outdoor mesocosm studies indicated single foliar applications of florpyrauxifen-benzyl provided >90% parrotfeather watermilfoil control 4 and 8 weeks after treatment (WAT) at rates ≥ 29.4 g a.i. ha^{-1}. Alligatorweed was less sensitive than parrotfeather watermilfoil at the same tested rates in both greenhouse and outdoor mesocosm environments, and repeat applications of florpyrauxifen-benzyl at 29.4 to 58.8 g a.i. ha^{-1} were necessary to achieve >94% alligatorweed control 12 WAT. Both parrotfeather watermilfoil and alligatorweed plants displayed signs of recovery when

exposed to the lowest herbicide rate (14.7 g a.i. ha^{-1}) evaluated. Therefore, operational florpyrauxifen-benzyl applications of < 29.4 g a.i. ha^{-1} are discouraged for herbicide resistance management. Future research should screen additional aquatic herbicides as potential tank mix partners for improving alligatorweed control longevity from a single florpyrauxifen-benzyl treatment. Water resource managers would additionally benefit from studies evaluating florpyrauxifen-benzyl foliar plus directed in-water application strategies to simulate common emergent plant control scenarios.

Introduction

Aquatic plant invasions interfere with municipal and ecosystem services worldwide (Hussner et al. 2017). Marginal plant incursions are particularly detrimental for habitat quality, and limit the growth of desirable native plants through resource competition and displacement (Hofstra and Champion 2010). Furthermore, these invasive species promote vector-borne disease habitat (Orr and Resh 1999), restrict recreation opportunity, and obstruct intakes for water consumption and hydropower generation (Durden et al. 1975, Clayton and Champion 2006). Within invaded regions, some of the worst non-native marginal plants are parrotfeather watermilfoil [*Myriophyllum aquaticum* (Vell.) Verdc.] and alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.] (Clayton 1996).

Native to South America, parrotfeather watermilfoil is a sprawling marginal plant often forming monospecific floating mats in quiescent waters (e.g., ditch banks, shallow wetlands, and littoral edges) (Wersal and Madsen 2011). The feather-like leaves, typical of *Myriophyllum* spp., appear in both emergent and submersed forms; thus providing parrotfeather watermilfoil with additional resource availability and greater tolerance to water level fluctuations (Sutton 1985, Wersal and Madsen 2010). Outside the native range, parrotfeather watermilfoil reproduction relies solely on plant fragmentation (Sutton 1985), with rhizome and rooting tissues ensuring perennial success (Sytsma and Anderson 1993). These physiologic characteristics allow parrotfeather watermilfoil the ability to invade diverse aquatic environments and thrive in poor growing conditions (e.g. drawdown events, high turbidity, eutrophic settings) (Maltchik et al. 2007, Wersal et al. 2013). Once established, parrotfeather watermilfoil can be difficult to eradicate with limited management tools or control tactics. At present, parrotfeather watermilfoil is widely distributed throughout the United States and New Zealand after plants escaped from the aquarium trade (Sutton 1985, Hofstra et al. 2006).

Alligatorweed is a marginal, stoloniferous plant in the Amaranthaceae family, native to South America (Julien et al. 1995). A perennial species, alligatorweed is characterized by vigorous aquatic growth with dense roots or as a free-floating entangled mat, and can inhabit both aquatic and terrestrial environments (Dugdale et al. 2010). Vegetative propagation lends to alligatorweed invasion success outside of its native range (Dugdale et al. 2010), because fragmented stems readily develop adventitious roots from the nodes that provide anchorage at new sites (Langland 1986). Early-stage invasion requires prompt management, as established alligatorweed populations are difficult to control (Hofstra and Champion 2010). Alligatorweed is distributed throughout the southeastern United States following release from ship ballast in the late 1800’s (Buckingham 1996), with localized dispersion found in the North Island of New Zealand since its incursion in the early 1900’s (Champion and Clayton 2000).

Effective control methods for parrotfeather watermilfoil and alligatorweed are limited in part because of the previously described plant physiology characteristics, but also because of the inherent challenges of developing methods that are effective across aquatic and wetted-terrestrial invasion sites. Biological control agents such as grass carp (*Ctenopharyngodon idella*) and beetles (flea beetle [*Lysathia ludoviciana*]; alligatorweed beetle [*Agasicles hygrophila*]), have shown promise (Julien et al. 1995, Cilliers 1999, Garner et al. 2013); however, the effectiveness of management relies heavily on localized infestation and environmental conditions (i.e., suitable insect overwintering temperatures and reproductive ability to maintain biocontrol populations). Similarly, biological control deployment is strictly governed and will not be suitable among regions in which permitting restricts release (Clayton 1996, Hussner et al. 2017). Mechanical methods for parrotfeather watermilfoil and alligatorweed control can offer short-term biomass reduction, but the long-term effectiveness of mechanical techniques is limited due to plant reproductive strategies with increased disturbance (i.e., plant fragments contributing to new populations). Therefore, the most frequently utilized management approach for controlling these marginal weed species in the United States and New Zealand is with herbicides (Wersal and Madsen 2007, Hofstra and Champion 2010, Dugdale and Champion 2012).

Though frequently relied on, invasive plant control with herbicides is also limited by both the efficacy and availability of registered products. For example, New Zealand has just two registered aquatic herbicides, diquat dibromide (photosystem I inhibitor) and endothall dipotassium salt (protein phosphatase inhibitor), although glyphosate (EPSP inhibitor), imazapyr (acetolactate synthase inhibitor), and metsulfuron-methyl (acetolactate synthase inhibitor) applications do occur under special-use permitting (Hofstra and Champion 2010, NZ EPA 2012). The United States has a broader herbicide portfolio, however not all available herbicides control parrotfeather watermilfoil and alligatorweed with consistency. Contact herbicides (e.g., diquat) are effective in providing short-term control of parrotfeather watermilfoil and alligatorweed, while systemic herbicides (e.g., glyphosate and imazapyr) often achieve longer periods of control (Wersal and Madsen 2007, Schooler et al. 2008, Hofstra and Champion 2010, Clements et al.

2014). The systemic herbicide imazapyr (acetolactate synthase inhibitor) at 584 and 1123 g active ingredient (a.i.) ha^{-1} achieved complete control of parrotfeather watermilfoil in a 10-week study (Wersal and Madsen 2007), while repeat applications of imazapyr at 0.16 to 0.64 kg a.i ha^{-1} were required for alligatorweed suppression (Hofstra and Champion 2010). In a mesocosm study, Cox et al. (2014) found triclopyr at 3.36 and 6.72 kg acid equivalent (a.e.) ha^{-1}, and 2,4-D (synthetic auxin) at 1.06 and 2.13 kg a.e. ha^{-1}, effectively reduced alligatorweed biomass >91%. Initial control of parrotfeather watermilfoil has also been achieved using similar rates of 2,4-D and triclopyr, though plant regrowth was reported to occur with single herbicide applications (Hofstra et al. 2006, Wersal and Madsen 2010). While herbicide options do exist for managing these marginal species, repeat applications are generally required to meet management goals. There is a clear need to investigate additional herbicides as they become available.

In 2018, florpyrauxifen-benzyl (synthetic auxin) was registered for aquatic site applications in the United States as two available formulations (emulsifiable [EC] and soluble [SC] concentrates). Florpyrauxifen-benzyl is considered a reduced risk herbicide (U.S. EPA 2017) and has ca. ~100 times lower use rate than the aquatic auxin herbicides, 2,4-D and triclopyr. In a 4-week study, initial dose-response screenings of florpyrauxifen-benzyl subsurface applications provided 50% effective concentration (EC_{50}) values of < 0.3 μg a.i. L^{-1} for parrotfeather watermilfoil, and 0.96 to 1.8 μg a.i. L^{-1} (Richardson et al. 2016). A similar study investigating concentration and exposure time (CET) relationships indicated two submersed Myriophyllum spp. were controlled 30 and 60-days after treatment (DAT) when exposed to florpyrauxifen-benzyl 3.0 μg a.i. L^{-1} for 6 to 24-hrs (Beets et al. 2019). Foliar applications of select herbicides are generally more effective (economically and ease of application) than submersed application methods when targeting marginal plant invaders. While in-water herbicide concentrations are not directly comparable to foliar application rates, no difference in control was observed when parrotfeather watermilfoil plants were treated with maximum label rates of triclopyr as subsurface (2.5 mg a.e. L^{-1}) and foliar (6.7 kg a.e. ha^{-1}) applications (Wersal and Madsen 2010).

Currently, there has been no efficacy data published describing foliar applications of florpyrauxifen-benzyl for parrotfeather watermilfoil or alligatorweed control, and there remains a need to evaluate the effectiveness of this herbicide applied as a foliar solution to target frequently managed marginal plant invaders and potentially broaden the available herbicide options. Since both species are susceptible to other aquatic auxin herbicides (i.e., 2,4-D and triclopyr), we hypothesize parrotfeather watermilfoil and alligatorweed will be sensitive to the tested florpyrauxifen-benzyl rates. The objective of this research was to evaluate foliar rates of florpyrauxifen-benzyl to determine the control of parrotfeather watermilfoil and alligatorweed for water resource management in the United States and New Zealand.

Materials and Methods

Greenhouse Study

Trials were conducted at the North Carolina State University, Aquatic Weed Control Labs (Raleigh, NC, USA) during a 4-wk study period and repeated in time (03 November and 05 December 2017, respectively). Alligatorweed and parrotfeather watermilfoil were propagated as 10 cm apical shoot tips and transplanted individually into 10 cm^2 (0.92 L) pots (i.e., one species shoot per pot) containing commercial potting media (Fafard® 2 Mix[1]). During transplant, Osmocote® slow-release fertilizer[2] (14-14-14) was incorporated into the planting media at a rate of 15 g per pot. Pots remained saturated throughout the experimental period via overhead mist irrigation system at 0.635 cm^{-1} of tap water, dispensed twice daily. Supplementary light was provided to simulate a photoperiod of 12-h per day^{-1} with ambient temperature maintained at 27±3 ºC. Plants were cultivated 14-28 days prior to treatment to allow for root establishment and allow for active shoot growth (1-2 shoots per pot; shoots measuring 16-24 cm above soil line at treatment).

Herbicide treatments included three foliar applications of florpyrauxifen-benzyl emulsifiable concentrate (EC)[3] [SLF-9523] (29.4, 44.1, and 58.8 g a.i. ha^{-1}), and a nontreated control. Treatments were arranged as a randomized complete block design with four replications. Treatments were applied using a pressurized CO_2 spray chamber with a single Teejet® XR8003 nozzle[4] at 234 L ha^{-1} to the foliage of the target species. Visual ratings of percent control were made on at two and four-weeks after treatment (WAT) on a scale ranging from 0% (no injury) to 100% (complete necrosis). Above-sediment plant biomass was harvested 4 WAT and dried at 60 °C for 48-hr prior to recording biomass (g dry mass).

Outdoor Mesocosm Study

Trials occurred at the National Institute of Water and Atmospheric Research (NIWA) Ruakura Campus, New Zealand on 10 January 2018 (New Zealand summer). Separate alligatorweed and parrotfeather watermilfoil experimental treatment containers were established using basal stem material (~10 to 15 cm in length; 15 stems per experimental unit) planted in 60 L plastic bins (bin surface area: 0.23 m^2), filled two-thirds with sediment, and covered with ~7 cm sand layer. Freshwater (pH 7.2) was added to each bin to occupy the additional space (~10 cm) in the mesocosm bin with water replenished throughout the study period. Plant establishment ensued 2-months prior to herbicide treatment to promote active shoot growth and increase foliage cover (bin surface area: >50% alligatorweed, and >80% parrotfeather watermilfoil). Treatment bins remained outdoors for the duration of the study with ambient temperature conditions of 18.5±2 ºC.

Using the 2017 NC greenhouse studies as a preliminary plant response reference, treatments included four foliar rates of the florpyrauxifen-benzyl EC formulation [SLF-9523] (14.7, 29.4, 44.1, and 58.8 g a.i. ha^{-1}), and a nontreated control. Treatments were arranged in a randomized complete block design with ten replications for both plant species. Hasten ESO[5] (esterified seed oil) was also included in the spray solution at 0.5% v/v during plant treatment. Applications were made using a handheld pump sprayer with an application volume of 234 L ha^{-1}. At treatment application, five nontreated bins representing each species were destructively harvested to determine pre-treatment biomass. Observations of auxin herbicide injury response and visual percent control estimates of exposed shoot material were evaluated throughout the study period as previously described for the greenhouse trials. At 6 WAT, alligatorweed regrowth occurred across all florpyrauxifen-benzyl rates tested, which initiated a retreatment to one-half of the replicates of the alligatorweed bins 8-weeks after initial treatment (8 WAIT) to evaluate the effectiveness between a single and repeat florpyrauxifen-benzyl foliar application. The study duration lasted 8-weeks for parrotfeather watermilfoil, and 12-weeks for alligatorweed. Plant shoot and root biomass was harvested at the end of each species study and dried at 60° C for 72 hrs to obtain biomass dry weights.

Statistical Analysis

Data from the greenhouse and mesocosm studies were subjected to analysis of variance (ANOVA), and Fisher's protected LSD post hoc test if significant effects ($P < 0.05$) were observed using RStudio[6] 'base', 'agricolae', and 'dplyr' packages (R Core Team 2020, de Mendiburu 2020, Wickham et al. 2021). A significant interaction was detected between run and species for the greenhouse study ($P < 0.001$), where nontreated plants in the second run were approximately 1.5x larger than the nontreated plants in the first run. Thus, biomass reductions for the greenhouse study were analyzed by run. All other data were pooled across runs for the greenhouse studies.

Results and Discussion

Greenhouse Study

Initial injury of parrotfeather watermilfoil and alligatorweed occurred within 24 to 48-hours after treatment (HAT) of florpyrauxifen-benzyl EC foliar applications, and appeared as terminal leaf and stem epinasty (twisting). Within 72 HAT, parrotfeather watermilfoil treated with florpyrauxifen-benzyl 29.4, 44.1, and 58.8 g a.i. ha^{-1} exhibited canopy collapse (wilting), chlorosis, and preliminary senescence. Alligatorweed also appeared chlorotic 72 to 96 HAT, with increased hyponasty (downward leaf convexity) when exposed to 44.1 and 58.8 g a.i. ha^{-1} rates of florpyrauxifen-benzyl.

All foliar rates of florpyrauxifen-benzyl rates provided >95% control of parrotfeather watermilfoil by 2-weeks after treatment (WAT) (Table 1). At 4 WAT, no visual control or plant biomass differences occurred as herbicide rate increased for parrotfeather watermilfoil (Figure 1). However, parrotfeather watermilfoil biomass reduction did differ between trial runs ($P < 0.05$), thus having a greater biomass reduction in the first trial run, which was likely attributed to initial biomass compared to run two. The average reduction in biomass compared to the nontreated control plants averaged 57.2 ± 3.7% and 46.2 ± 5.8% in run one and two, respectively.

Alligatorweed was less sensitive to florpyrauxifen-benzyl treatments than parrotfeather watermilfoil; where visual control estimates were 72.5 and 31.9% less for alligatorweed than parrotfeather watermilfoil at the 58.8 g a.i. ha^{-1} rate (2 and 4 WAT assessments, respectively). Increased herbicide rate did result in greater alligatorweed injury (e.g., basal stem splitting and necrosis) from 2 to 4 WAT (Table 1). At 4 WAT, alligatorweed control with the 58.8 g a.i. ha^{-1} herbicide rate was different from the 29.4 and 44.1 g a.i. ha^{-1} florpyrauxifen-benzyl rates; however, there was no difference in alligatorweed control between the 29.4 and 44.1 g a.i. ha^{-1} rates at 4 WAT (Table 1). Conversely, there was no difference in alligatorweed biomass reduction at harvest (4 WAT) across the florpyrauxifen-benzyl rates tested (Figure 1). Similar to the parrotfeather watermilfoil studies, alligatorweed biomass reduction differed between runs ($P < 0.05$), with greater biomass reduction occurring in the first run. Treated plants averaged 63.1 ± 4.1% and 36.6 ± 8.1% in runs 1 and 2, respectively at harvest. Nonetheless, alligatorweed treated with florpyrauxifen-benzyl at 29.4 and 44.1 g a.i. ha^{-1} exhibited recovery in the form of new shoots emerging from the root crown compared to plants treated with 58.8 g a.i. ha^{-1}, which suggests plant control improved as the florpyrauxifen-benzyl rate increased.

Outdoor Mesocosm Study

Based on greenhouse evaluations of parrotfeather watermilfoil sensitivity to florpyrauxifen-benzyl (all rates produced injury within 24 HAT and >92% control 4 WAT), the outdoor mesocosm study included an additional lower herbicide dose (14.7 g a.i. ha^{-1}; half the previously tested low rate) to further determine plant sensitivity to the new auxin herbicide. Similar to the greenhouse study, parrotfeather watermilfoil exhibited a more rapid response to florpyrauxifen-benzyl than did alligatorweed (Table 2). Within 48 HAT, parrotfeather watermilfoil displayed apical epinasty and plant canopy collapse among all treated mesocosms. Plants treated with florpyrauxifen-benzyl at 44.1 or 58.8 g a.i. ha^{-1}, reached complete necrosis by 2 WAT. Following the 2 WAT evaluation, visual control estimates remained >96%, with all florpyrauxifen-benzyl rates providing >99% control 8 WAT (Table 2). All herbicide rates reduced parrotfeather watermilfoil shoot and root biomass (Figure 2). However, signs of recovery were present for plants treated with the 14.7 g a.i. ha^{-1} rate, with minor regrowth of submersed plant tissue discovered at the sediment interface during harvest (8 WAT). Wersal and Madsen (2010) described similar parrotfeather watermilfoil regrowth forming at the root crowns following a foliar application of the auxin herbicide triclopyr (6.7 kg a.e. ha^{-1}). A potential sub-lethal response among florpyrauxifen-benzyl 14.7 g a.i. ha^{-1} treatments in this study should be further evaluated to determine if recovery of treated parrotfeather watermilfoil may occur with longer study periods (e.g., >12-weeks) to evaluate control longevity.

Initial injury observations of alligatorweed under outdoor mesocosm settings corresponded to the early findings of plant sensitivity to florpyrauxifen-benzyl with the greenhouse experiments. Signs of herbicide injury such as leaf hyponasty first appeared on plants treated with the 58.8 g a.i. ha^{-1} rate by 48 HAT, and all treatments experienced varying levels of chlorosis 5 DAT (leaf chlorosis increasing with florpyrauxifen-benzyl dose). Visual estimates of control peaked 4 WAT across all rates, with 44.1 and 58.8 g a.i. ha^{-1} providing >90% control. Alligatorweed began to recover 5 to 6 WAT, with plants showing signs of recovery typical of auxin herbicide injury (e.g., thin and elongated leaves, swollen nodes, cupped leaves, and witches broom). Plants that received a single herbicide application, regardless of original rate, were controlled <18% based on visual control 12 WAIT, while repeat applications of florpyrauxifen-benzyl at 29.4, 44.1, and 58.8 g a.i. ha^{-1} provided >94% control 12 WAIT. At harvest, all treatments reduced shoot biomass regardless of rate ($P < 0.001$; Figure 3). While repeat applications of florpyrauxifen-benzyl at 29.4, 44.1, and 58.8 g a.i. ha^{-1} further reduced biomass, there were no significant differences among initial and repeat applications ($P > 0.05$).

However, the 58.8 g a.i. ha^{-1} treatments (initial and repeat) were the most effective at reducing alligatorweed shoot biomass 12 WAIT (81.8% and 77.6%, respectively). Conversely, single and repeat florpyrauxifen-benzyl 14.7 g a.i. ha^{-1} treatments failed to provide acceptable plant control, as biomass increased over the study period (Figure 3).

Previous studies from the United States and New Zealand demonstrated that the auxin herbicides 2,4-D and triclopyr effectively controlled parrotfeather watermilfoil in small-scale mesocosm and field settings (Hofstra et al. 2006, Gray et al. 2007, Wersal and Madsen 2010). However, foliar applications of 2,4-D and triclopyr have resulted in variable alligatorweed control. One mesocosm study found foliar applied 2,4-D (1.06 and 2.13 kg a.e. ha^{-1}) and triclopyr (3.36 and 6.72 kg a.e. ha^{-1}) effectively reduced alligatorweed biomass (91 to 94% and 95%; 2,4-D and triclopyr, respectively) 12 WAT (Cox et al., 2014). Similarly, triclopyr (3.2 to 13.0 kg a.e. ha^{-1}) reduced alligatorweed biomass on recently established plants in a mesocosm experiment; however, a repeat application was necessary to maintain effective control when plants were more mature and had been established 8 MAT (Hofstra and Champion 2010). Likewise, a single foliar application of 2,4-D provided 80% alligatorweed control 2 WAT when sprayed in a shallow drainage canal in North Carolina with a 0.46 g a.e. 100 L^{-1} solution, but did not provide control 8 WAT (Langland 1986).

While both parrotfeather watermilfoil and alligatorweed exhibit sensitivity to auxin herbicides, poor herbicide translocation from foliar applied herbicides likely contributes to reduced alligatorweed control longevity (Dugdale and Champion 2012). Haug et al. (2021) evaluated the absorption rates of several submersed plant species and discovered Eurasian watermilfoil (*Myriophyllum spicatum* L.), hybrid watermilfoil (*Myriophyllum spicatum* L. x *Myriophyllum sibiricum* Komarov), and variable watermilfoil (*Myriophyllum heterophyllum*

Michx.) treated with radiolabeled florpyrauxifen-benzyl at 10 µg a.i. L^{-1} had rapid active ingredient uptake in the plant shoots (24.2, 5.9, and 92.7 HAT to reach 90% shoot absorption, respectively). Likewise, a similar ^{14}C study discovered basipetal translocation of the auxin herbicide, 2,4-D, when foliar applied to mature emergent parrotfeather watermilfoil leaves (Sutton and Bingham 1970). In a small-scale CET study, Mudge et al. (2021) noted Eurasian watermilfoil was completely controlled 5 WAT following subsurface florpyrauxifen-benzyl applications of 3, 6, or 9 µg a.i. L^{-1} at 0.5, 1, or 3 hr exposure times. While not directly comparable, these past studies further corroborate our findings where parrotfeather watermilfoil, as a Myriophyllum spp., displayed greater sensitivity to foliar applied florpyrauxifen-benzyl than observed for alligatorweed. Richardson et al. (2016) noted florpyrauxifen-benzyl in-water activity for submersed alligatorweed in small mesocosms had greater control (EC_{50} of 0.96 to 1.8 µg L^{-1}) than commonly expected among 2,4-D or triclopyr applications. Since absorption and translocation of foliar-applied herbicides like glyphosate and imazapyr are limited within alligatorweed (Bowmer et al. 1993, Tucker et al. 1994), plants partially submersed in standing water would likely have even less control compared to plant targets having completely exposed biomass. It is unknown if alligatorweed foliar application studies from New Zealand trials experienced reduced efficacy having 10 to 20% of the basal portions of the plants submersed. Since florpyrauxifen-benzyl does show some in-water activity on alligatorweed (Richardson et al. 2016), foliar herbicide spray not retained on the plant foliage may have accumulated in the ~10 cm of standing water and made available for plant absorption. Future studies should determine florpyrauxifen-benzyl absorption and metabolism in alligatorweed, and foliar plus in-water application techniques to evaluate the translocation of the florpyrauxifen-benzyl molecule when applied to submersed and foliar plant material.

In conclusion, this small-scale research demonstrates florpyrauxifen-benzyl has potential for parrotfeather watermilfoil control, with similar use patterns to other auxin herbicides for alligatorweed management. Results from both greenhouse and outdoor mesocosm studies indicate florpyrauxifen-benzyl has value in controlling parrotfeather watermilfoil with >90% control 4 and 8 WAT, at rates $\geq$ 29.4 g a.i. ha^{-1}. Applying the lowest rate (14.7 g a.i. ha^{-1}) evaluated in this study under operational conditions would likely result in survival for both parrotfeather watermilfoil and alligatorweed. This application rate could also select for herbicide-resistant biotypes within a population (Richardson 2008), and is strongly discouraged. While there was no difference in alligatorweed biomass reduction between florpyrauxifen-benzyl applied at 29.4 to 58.8 g a.i. ha^{-1} single or repeat applications, a repeat application would be necessary to achieve >90% alligatorweed control 12 WAT based on results of these studies. Currently, repeat applications are common practice for long-term alligatorweed management and eradication programs (Hofstra and Champion, 2010).

Florpyrauxifen-benzyl is registered in the United States, and early operational use of the herbicide has shown favorable outcomes for controlling parrotfeather watermilfoil and alligatorweed (SN Sardes, personal communication). If approved in the country in the future, florpyrauxifen-benzyl may broaden the current herbicide portfolio in New Zealand where aquatic herbicide selection is limited. Future work should evaluate selectivity of this herbicide against potentially sensitive shoreline species (e.g., *Polygonaceae* spp.) so that use patterns do not negatively affect non-target plant communities. Operational implementation of florpyrauxifen-benzyl in field settings should designate seasonal herbicide use patterns for parrotfeather watermilfoil and alligatorweed management (e.g., application timing and plant establishment), potential differences in plant control between product formulations (EC vs. SC), and the appropriate contact times needed for effective control (e.g., concentration and exposure trials simulating fetch wash). Water resource managers would additionally benefit from foliar combination studies with additional herbicide modes of action to screen potential tank mix partners for improving control longevity for alligatorweed and resistance management.

Sources of Materials

[1]Fafard® 2 Mix potting media, Conrad Fafard, Inc., P.O. Box 790, Agawam, MA 01001.

[2]Osmocote® fertilizer, The Scotts Company, 14111 Scottslawn Road, Marysville, OH 43040.

[3]ProcellaCOR EC, SePRO Corporation, Carmel, IN 46032.

[4]Teejet® XR8003 flat-fan nozzle, TeeJet Technologies, P.O. Box 832 Tifton, GA 31794.

[5]Hasten™ ESO Spray Adjuvant, BASF New Zealand Limited, P.O. Box 407, Auckland 1140.

[6]R version 4.0.3, R Foundation for Statistical Computing, Vienna, Austria.

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TABLES

Table 3-1. Visual estimates of parrotfeather watermilfoil and alligatorweed control following foliar applications of florpyrauxifen-benzyl in the North Carolina greenhouse study.

	Parrotfeather		Alligatorweed	
Treatment Rate	2 WAT[a]	4 WAT	2 WAT	4 WAT
g ai ha^{-1}	----------------------%----------------------[b,c]			
29.4	95.6 a	92.3 a	23.1 a	56.3 a
44.1	95.6 a	97.5 a	21.9 a	59.4 a
58.8	98.8 b	100.0 a	26.3 a	68.1 b
Nontreated Control	0.0 c	0.0 b	0.0 b	0.0 c

[a]WAT: weeks after treatment.

[b]Control ratings based on visual estimates 0% (no injury) to 100% (complete desiccation).

[c]Means within columns followed by the same letter do not differ according to Fisher's protected LSD ($P < 0.05$, n= 4).

Table 3-2. Visual estimates of parrotfeather watermilfoil and alligatorweed control following foliar applications of florpyrauxifen-benzyl in the New Zealand outdoor mesocosm study.

	Parrotfeather			Alligatorweed			
Treatment Rate[a]	2 WAT[b]	4 WAT	8 WAT	2 WAT	4 WAT	8 WAT	12 WAIT
g ai ha^{-1}	--%--[c,d]						
14.7 (Single)	96.3 a	98.7 a	99.2 a	47.5 a	56.3 a	5.5 a	0.0 a
29.4 (Single)	99.7 a	99.9 b	99.7 a	77.5 b	85.4 b	49.5 b	2.0 a
44.1 (Single)	100.0 a	99.9 b	100.0 a	78.3 b	90.7 b	62.4 bc	10.0 ab
58.8 (Single)	100.0 a	100.0 b	100.0 a	85.4 b	93.8 b	68.3 c	18.0 b
14.7 (Repeat)	-----	-----	-----	-----	-----	-----	55.0 c
29.4 (Repeat)	-----	-----	-----	-----	-----	-----	94.0 d
44.1 (Repeat)	-----	-----	-----	-----	-----	-----	95.6 d
58.8 (Repeat)	-----	-----	-----	-----	-----	-----	98.2 d
Nontreated Control	0.0 b	0.0 c	0.0 b	0.0 c	0.0 c	0.0 a	0.0 a

[a]All treatments included the addition of esterified seed oil (ESO) at 0.5% v/v in the spray solution.

[b]WAT: weeks after treatment; WAIT: weeks after initial treatment (4 weeks after repeat applications for select alligatorweed bins).

[c]Control ratings based on visual estimates 0% (no injury) to 100% (complete desiccation).

[d]Means within columns followed by the same letter do not differ according to Fisher's protected LSD ($P < 0.05$, $n = 10$).

FIGURES

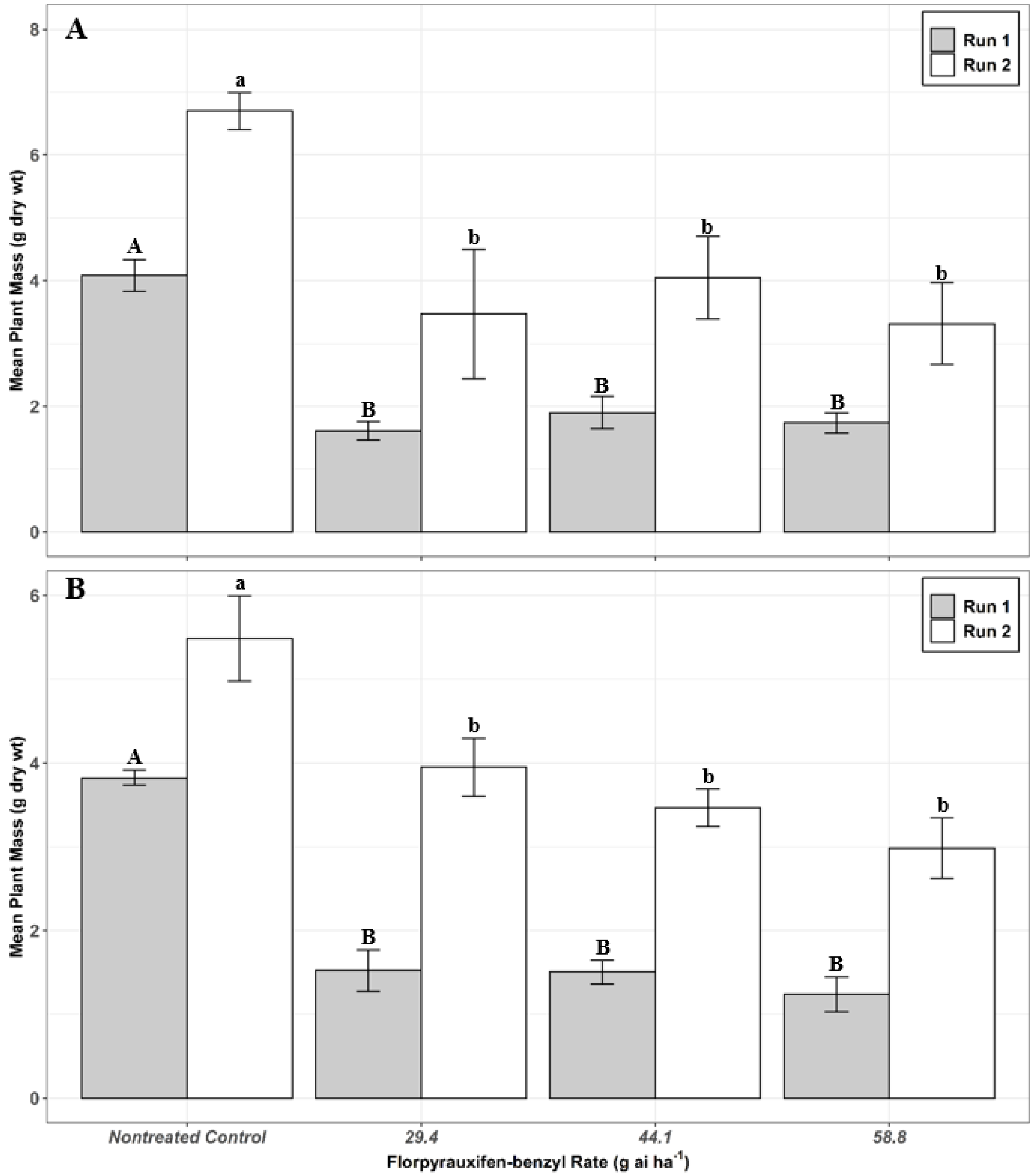

Figure 3-1. North Carolina greenhouse study response of (A) parrotfeather watermilfoil and (B) alligatorweed four weeks after treatment with florpyrauxifen-benzyl. Graphs show dry weights (mean g dry weight ± SE). Treatments within runs that share the same letter are not different using Fisher's LSD at P < 0.05 significance level (n = 4).

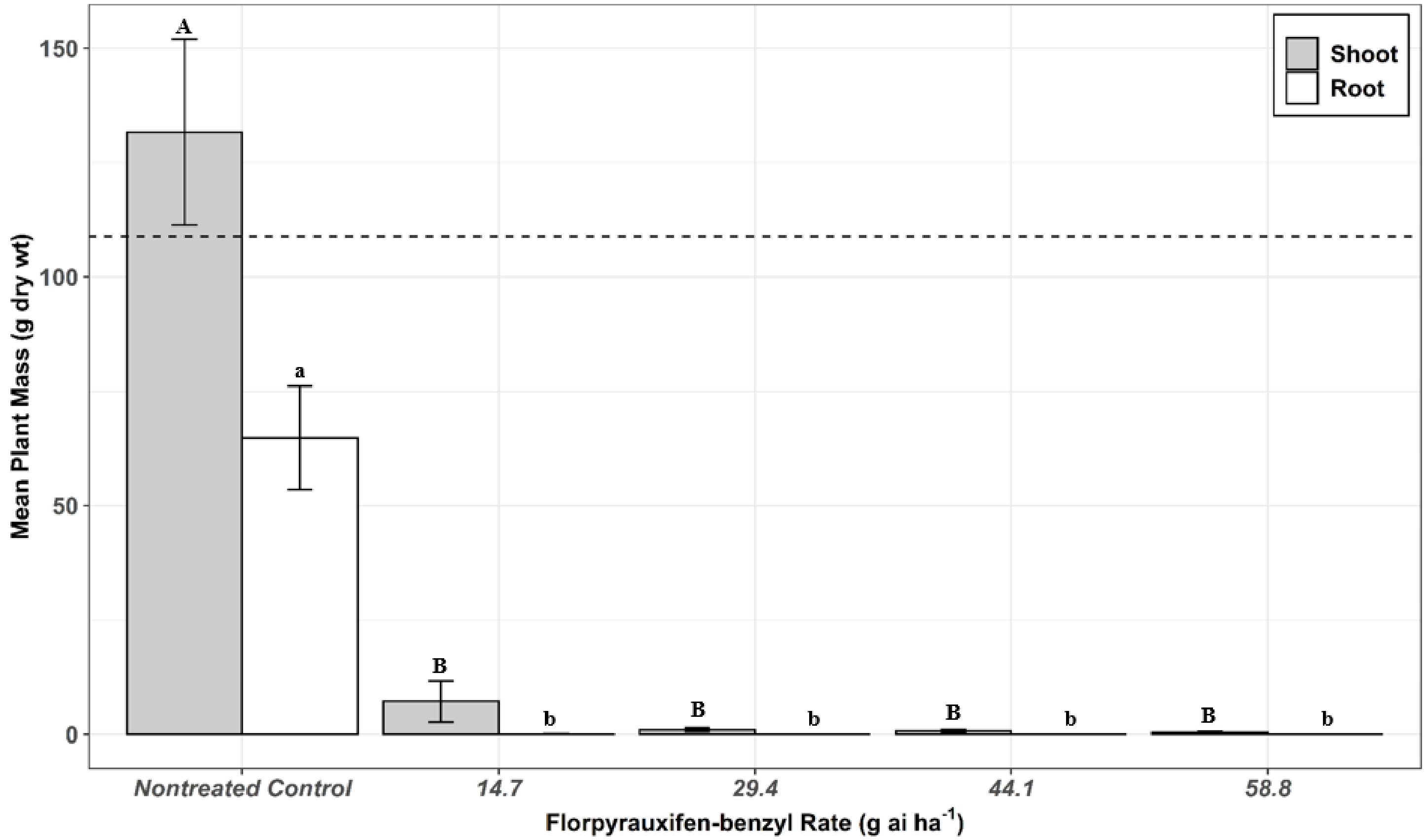

Figure 3-2. Response of parrotfeather watermilfoil shoot and root dry weights (mean g dry weight ± SE) to foliar-applied florpyrauxifen-benzyl at eight weeks after treatment in outdoor mesocosms in New Zealand. Treatments that share the same letter are not different using Fisher's LSD at $P < 0.05$ significance level ($n = 10$). The horizontal dash line represents mean pretreatment shoot biomass. All parrotfeather watermilfoil nontreated control plants actively grew over the study duration compared to the pretreatment reference (nontreated foliar biomass increased 20.9%).

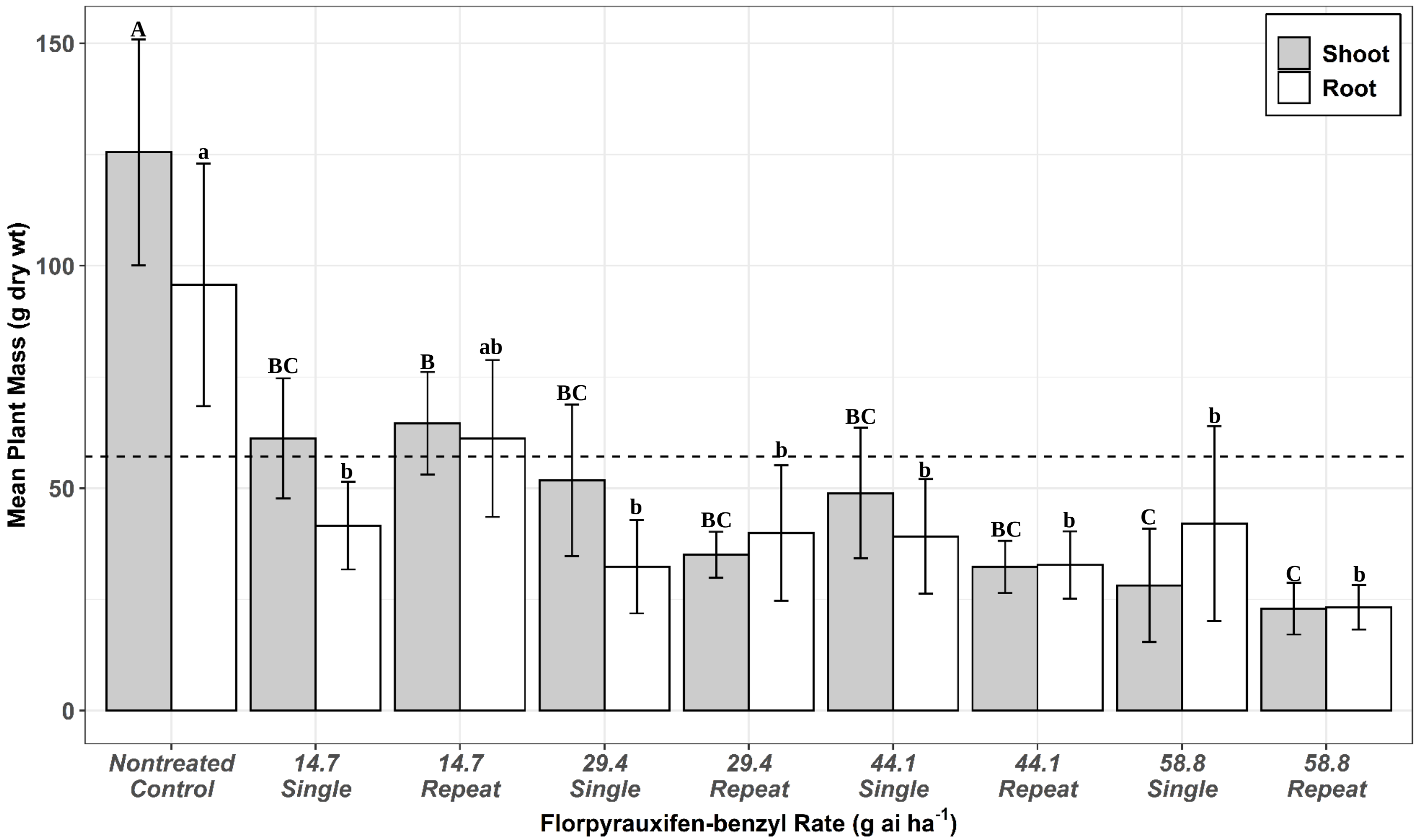

Figure 3-3. Response of alligatorweed shoot and root dry weights (mean g dry weight ± SE) to foliar-applied florpyrauxifen-benzyl at twelve weeks after treatment in outdoor mesocosms in New Zealand. Treatments that share the same letter are not different using Fisher's LSD at $P < 0.05$ significance level ($n = 10$). The horizontal dash line represents mean pretreatment shoot biomass. All alligatorweed nontreated control plants actively grew over the study duration compared to the pretreatment reference (nontreated foliar biomass increased 67.6%).

CHAPTER 4: Susceptibility of native and invasive submersed plants in New Zealand to florpyrauxifen-benzyl in growth chamber exposure studies

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Howell AW, Hofstra DE, Heilman MA, Richardson RJ (2022) Susceptibility of native and invasive submersed plants in New Zealand to florpyrauxifen-benzyl in growth chamber exposure studies. Invasive Plant Sci Manag

Abstract

Invasive aquatic plants constantly threaten freshwaters and associated environs globally. Water resource managers frequently seek new control tactics to combat invasive macrophytes, especially when the availability of herbicides registered for submersed plant control is limited. The synthetic auxin herbicide, florpyrauxifen-benzyl, recently registered (2018) for aquatic site applications in the United States, has shown success in controlling several invasive aquatic weeds. Studies were conducted to evaluate responses of native and invasive submersed plants to florpyrauxifen-benzyl under growth chamber conditions to provide insight on the selectivity of varying herbicide concentrations in New Zealand. Florpyrauxifen-benzyl concentrations evaluated ranged from 0.01 to 107.86 µg ai L^{-1}, encompassing the maximum use concentration (48 µg L^{-1}) for submersed plant applications. Dose–response metrics indicated the New Zealand native species watermilfoil [*Myriophyllum triphyllum* Orchard] was highly sensitive to florpyrauxifen-benzyl following a 21-d static exposure, having a dry weight 50% effective concentration (EC_{50}) value of 1.2 µg L^{-1}. The invasive species oxygen-weed [*Lagarosiphon major* (Ridley) Moss] and Canadian waterweed [*Elodea canadensis* Michx.] were less sensitive,

with dry weight EC_{50} values of 35.4 and >107.86 µg L^{-1}, respectively. Brazilian waterweed [*Egeria densa* Planch.] was most tolerant to the tested concentrations, as EC_{50} values were not achieved. Overall, results indicate florpyrauxifen-benzyl demonstrates potential for controlling *L. major*, with further large-scale screening required to confirm control among field site applications. As the native species (*M. triphyllum*) was most sensitive to florpyrauxifen-benzyl compared with the invasive plant evaluated (I/N ratio indicated >31.3 times more sensitive), any targeted concentration used for invasive plant control for field applications would likely injure the native *M. triphyllum* plants. Future studies should investigate additional native and invasive species for management guidance and consider how exposure times influence plant response using similar florpyrauxifen-benzyl concentrations tested in the present study.

Management Implications

Since their introduction in the early 1900s, invasive submersed plants have adversely affected lakes and waterways on both islands of New Zealand. Some of the worst plant invaders in the country are from the "oxygen weed" (Hydrocharitaceae) family and yield high biomass plant canopies that displace native flora and fauna and obstruct the recreational use and economic activities in affected lakes. Water resource managers regularly utilize herbicides to control aquatic weeds to restore invaded lakes and waterways. However, there are only two aquatic herbicides currently registered in New Zealand for submersed weed control, which limits the scope of management opportunity. Registration of the synthetic auxin herbicide, florpyrauxifen-benzyl, in the United States has provided another chemical option for aquatic weed control. However, limited data are available for florpyrauxifen-benzyl concentrations required to effectively control frequently managed Hydrocharitaceae in New Zealand. A dose–response study was conducted to examine the sensitivity of three New Zealand invasive species oxygen-weed; [*Lagarosiphon major* (Ridley) Moss, Brazilian waterweed*; Egeria densa* Planch., and Canadian waterweed; *Elodea canadensis* Michx.] and one native submersed plant watermilfoil (*Myriophyllum triphyllum* Orchard) to the herbicide. Among early invasion scenarios, native plants are frequently found cohabitating with invasive weed species. Therefore, herbicides that provide selective control with minimal impact to native plant species are desired. Following the 21-d growth chamber evaluations, we found the native plant *M. triphyllum* to be the species most sensitive to herbicide. The invasive plant *L. major* was also sensitive to florpyrauxifen-benzyl. The invasive plants *E. canadensis* and *E. densa* displayed a sublethal response from herbicide, and control was not achieved at any florpyrauxifen-benzyl concentration labeled for submersed plant applications (48 µg ai L^{-1}). Therefore, targeted concentrations deployed for invasive plant control within mixed communities would likely injure the native *Myriophyllum* spp. However, native species do recover from seedbanks following invasive plant removal. Future research should evaluate additional native and introduced invasive species for best management guidance in New Zealand and investigate approaches, including concentration and exposure time relationships, to provide effective control of submersed aquatic weeds.

Introduction

Preservation of native submersed aquatic plants is vital for conserving biodiversity within waterways (Hofstra et al. 2021; Kovalenko et al. 2010), as macrophytes are essential for numerous ecosystem services (Carpenter and Lodge 1986; Cyr and Downing 1988; Madsen et al. 2001; Petr 2000; Valley et al. 2004). Conversely, the intrusion of invasive submersed plants into aquatic biomes can displace endemic flora and fauna through structural and resource competition resulting from the formation of aggressive monotypic stands that limit light, carbon, and nutrient availability (Hofstra et al. 2018; True-Meadows et al. 2016; Wells et al. 1997). Similarly, invasive submersed macrophytes that produce high biomass yielding canopies (e.g., Hydrocharitaceae) commonly obstruct navigation, clog water intakes for irrigation and hydroelectric generation, and impede recreation and economic opportunities (Carpenter and Lodge 1986; Clayton and Champion 2006; Langeland 1996). In New Zealand, some of the most problematic invasive submersed plants are known as the "oxygen weeds" (Clayton 1996), which includes the species oxygen-weed [*Lagarosiphon major* (Ridley) Moss], Canadian waterweed [*Elodea canadensis* Michx.], and Brazilian waterweed [*Egeria densa* Planch.]. Water resource managers regularly seek effective control methods to eradicate or manage invasive aquatic plants to enable the recovery of desirable native habitats.

While biological, physical, and mechanical control options do exist, herbicides are largely the most economic, effective, and selective management tool utilized to control invasive aquatic weeds (Hussner et al. 2017; Muller et al. 2021). It is important to recognize that applying herbicides for aquatic weed management requires several factors to be considered, some of which include regulatory and economic constraints, herbicide efficacy and selectivity, and public support for the treatment (Champion et al. 2002; Clayton 1996; Madsen 2000; Stallings et al. 2015). Combinations of these factors influence initiatives used to broaden application strategies for restoring native plant populations within invaded waterways (Getsinger et al. 2008; Weber et al. 2020. When considering herbicide-based management strategies, it is critical to understand target and non-target plant responses. Further, these data will help to provide appropriate recommendations for management action in mixed assemblages of native and invasive species, especially because most available herbicides do not provide uniform control of aquatic weeds.

Currently, only two herbicides are labeled for submersed aquatic plant control in New Zealand, diquat-dibromide (WSSA Group 22; photosystem I inhibitor) and endothall dipotassium salt (WSSA Group 31; protein phosphatase inhibitor). For perspective, 16 herbicides are presently labeled for aquatic weed management in the United States (Gettys et al. 2020). A limited herbicide portfolio restricts management options and prompts selection pressures, which can select for herbicide-resistant plant populations that further complicate future invasive plant control (Richardson 2008). While endothall dipotassium salt and diquat-dibromide effectively control *L. major* (Wells and Champion 2010; Wells et al. 2014), only diquat-dibromide is efficacious on *E. canadensis* and *E. densa* (Glomski et al. 2005; Hofstra and Clayton 2001; Sesin et al. 2018; Skogerboe et al. 2006). Previous studies have examined additional herbicide sites of action (SOAs) including triclopyr (WSSA group 4; synthetic auxin), dichlobenil (WSSA Group 29; cellulose synthase inhibitor), and fluridone (WSSA Group 12; phytoene desaturase inhibitor) to control invasive submersed plants; however, these herbicides are not efficacious on *L. major*, *E. canadensis*, and *E. densa* in mesocosm or field studies (Hofstra and Clayton 2001; Wells et al. 1986), nor are these herbicides registered for aquatic weed control applications in New Zealand (Champion et al. 2019). While endothall dipotassium salt has shown some selectively on desirable native plants in the United States and New Zealand (Hofstra and Clayton 2001; Skogerboe and Getsinger 2002), diquat-dibromide is a nonselective contact herbicide, and applications are prone to off-target injury to native plants. Wells and Champion (2010) have suggested diquat had transient injury on native charophytes in New Zealand following invasive plant eradication efforts, though recovery was not immediate. Nevertheless, there remains a need to evaluate new herbicides as they become available to enhance current invasive aquatic plant management programs, while protecting native plant species like watermilfoil [*Myriophyllum* *triphyllum* Orchard], which may be frequently intermixed or adjacent to invasives (Rattray et al. 1994).

Florpyrauxifen-benzyl (WSSA Group 4; synthetic auxin) is a relatively new herbicide initially introduced for rice (*Oryza sativa* L.) production (Epp et al. 2016) and registered for aquatic use in the United States in 2018. Synthetic auxins have been utilized for crop and non-crop weed management since development in the 1940s (Peterson et al. 2016). This class of herbicides is frequently cited for their favorable management characteristics compared with other herbicide SOAs (Glomski and Netherland 2010; Grossmann 2010; Heap 2022; Sprecher et al. 1998). Synthetic auxin herbicides like florpyrauxifen-benzyl are unique in both selectiveness and phloem mobility within susceptible plants, as they mimic the natural plant growth hormone indole-3-acetic acid (Epp et al. 2016). Endogenous auxin compounds are essential for plant cell elongation and division, phototropism, apical dominance, and additional developmental processes (Gaines 2020; Grossmann 2010). Susceptible plants treated with synthetic auxins undergo rapid growth complexes when transcription factor proteins responsible for plant regulation become overwhelmed, triggering uncontrolled gene expression (Grossmann 2010; McCauley et al. 2020; Parry et al. 2009). Eventually, the process of uncontrolled gene expression initiates abscisic acid, hydrogen peroxide, and ethylene accumulation, leading to leaf senescence, cell death, and loss of turgor through multifaceted processes that are still undergoing investigation (Grossmann 2010; McCauley et al. 2020). Synthetic auxin overload in susceptible plants characteristically results in apical epinasty, twisting, and curling of leaf tissues. In the United States, florpyrauxifen-benzyl has provided an additional herbicide for selective invasive aquatic plant management among several common invasive weeds including hydrilla [*Hydrilla verticillata* (L.f.) Royle], watermilfoils [*Myriophyllum* spp.], and crested floatingheart

[*Nymphoides cristata* (Roxb.) Kuntze.] while having limited activity on native *Potamogeton* spp. (Beets and Netherland 2018; Mudge et al. 2021; Netherland and Richardson 2016; Richardson et al. 2016; Sperry et al. 2021). Additionally, florpyrauxifen-benzyl was classified by the U.S. Environmental Protection Agency as a reduced-risk herbicide (USEPA 2017) with favorable toxicological profiles (Buczek et al. 2020).

Given the limited number of registered herbicides in New Zealand, there remains a need to evaluate additional selective herbicides that provide different SOAs than those currently registered. Registration of such a herbicide would promote herbicide stewardship and increase treatment options for controlling invasive submersed plants. The objective of this study was to implement a small-scale screening method for evaluating relative sensitives to florpyrauxifen-benzyl of native and invasive plant species found in New Zealand using dose–response protocols. Based on previous screening studies (e.g., Beets et al. 2019; Howell et al. 2021; Netherland and Richardson 2016; Richardson et al. 2016), we hypothesize the native species, *M. triphyllum*, will be the most sensitive to the herbicide; however, we anticipate the invasive species tested to display comparable sensitivity to florpyrauxifen-benzyl.

Materials and Methods

A growth chamber experiment was conducted at the National Institute of Water and Atmospheric Research (NIWA) Ruakura Campus, Hamilton, New Zealand in fall (April to May) 2018. The experimental design closely followed a modified version of the Organisation for Economic Co-operation and Development (OECD) protocol described by Netherland and Richardson (2016) to evaluate the sensitivity of *M. triphyllum*, *L. major*, *E. canadensis*, and *E. densa* to florpyrauxifen-benzyl. Tested species were collected from on-site stock tanks or field harvested locally within the Waikato basin in March and April 2018. Plant species were then propagated in aerated outdoor tanks under ambient environmental conditions (μ = 18.5 to 20.0 C) with 50% shade fabric and monitored twice weekly to ensure adequate population vigor before testing.

At experiment initiation, apical shoot tips (6 cm) of each species were removed from the outdoor tanks. The basal portions of the apical shoots were secured with lead weights to ensure submersion during propagation. Weighted plant shoots were then placed in aerated 6-L bins containing dechlorinated tap water. Bins were subsequently placed in the growth chamber and monitored for 7 to 10 d for confirmation of plant root generation and shoot elongation. Growth chamber conditions were set to a constant 16-h light:8-h dark photoperiod, 21.5 C temperature, and light intensity of 130 to 160 μE m^{-2} s^{-1} at bench level. Following rooting confirmation, one shoot of each species was planted in a 20-ml vial filled with 16 cm of washed sand (i.e., a single shoot per vial). At minimum, 3 cm of the shoot was buried in the sand. Seven days before treatment, 1-L jars were filled with 750 ml of Smart and Barko solution, and each jar was provided with supplemental aeration (Smart and Barko 1985). Vials containing plants were then placed in the respective treatment vessels. Prior studies noted *Myriophyllum* spp. to be highly sensitive to florpyrauxifen-benzyl (Netherland and Richardson 2016; Richardson et al. 2016). As such, *M. triphyllum* plants and the Hydrocharitaceae evaluated in this study were isolated in separate treatment jars to evaluate herbicide concentration response endpoints based on the recognized sensitivity. Stock solutions of florpyrauxifen-benzyl suspension concentrate (ProcellaCOR SC, SePRO, Carmel, IN, USA) were produced for treatment and injected into the water column to achieve desired serial dose–response concentrations. Treatments consisted of static exposure to a geometric series of rates ranging from 0 to 107.86 µg ai L^{-1} (Table 1). Pretreatment water pH was 8.2 (SD ± 0.2) and temperature was 22.2 C (SD ± 1.4). All treatments were replicated five times (one jar was considered as one replication) following a randomized complete block design, and experiments were repeated in time (two consecutive runs). At treatment, five nontreated jars were removed to determine pretreatment weight and shoot length of each species. Trials lasted 21 d, and dechlorinated water was added to the jars as water loss occurred. Visual observations of auxin herbicide symptoms (e.g., chlorosis, epinasty, leaf-shattering) were documented throughout experimentation. At 21 d after treatment (DAT), above-sediment green plant tissue was harvested and blotted dry with paper towels, and then fresh weight (g) and shoot length (cm) were immediately recorded. Plants were then oven-dried at 60 C for 72 h to obtain a constant dry weight. Plant dry weights were measured using an analytical-grade balance with 0.001 g accuracy.

Statistical Analysis

There was no significant run effect according to ANOVA ($P > 0.05$), so treatment data were pooled over experiments to account for inherent response variability in the growth chamber studies. Plant shoot length, fresh biomass, and dry biomass metrics were transformed to percent inhibition (%In) of the nontreated control to standardize plant response to tested florpyrauxifen-benzyl concentrations tested using Equation 1:

$$\%\mathrm{In} = [(\mu_c - \mu_t)/\mu_c] \times 100 \qquad [1]$$

Where (μ_c) is the mean of the nontreated group and (μ_t) is the mean value of the treatment group. Percent inhibition (%In) was limited to the logical extremes (0% to 100%) to achieve appropriate parameters for modeling plant response to herbicide concentrations tested, as plant inhibition cannot physically exceed a 100% threshold. Following similar statistical procedures as Netherland and Richardson (2016), nonlinear regression analyses were performed in SigmaPlot v. 14.0 (SigmaPlot v. 14.0.3.192, Systat Software, Point Richmond, CA, USA) to estimate 50%

effective dose concentrations (EC_{50}). Equation 2 is the four-parameter log-logistic regression curve used to estimate EC_{50} shoot length and dry weight inhibition metrics at tested florpyrauxifen-benzyl concentrations as described in detail by Ritz et al. (2015) and Seefeldt et al. (1995).

$$Y = y_o + \{a/[1+(x/x_{EC50})^b]\} \qquad [2]$$

For Equation 2, the parameters y_o and a represent the limit extreme and difference values, b is the slope of the inflection point, x is the herbicide concentration, and x_{EC50} is the herbicide concentration providing 50% inhibition of the maximum (i.e., 100% In). Equation 3 is the Weibull four-parameter model used to estimate EC_{50} fresh weight inhibition metrics at tested florpyrauxifen-benzyl concentrations as described in detail by Price et al. (2012) and Brown and Mayer (1988).

$$Y = a \times \{1 - \exp[-(x - x_{EC50} + b + \ln(2)^{1/c}))/b)^c]\} \qquad [3]$$

For Equation 3, the parameter a is the upper asymptote, b is the slope of the inflection point, c is the shape of the curve, x is the herbicide concentration, and x_{EC50} is the herbicide concentration providing 50% inhibition of the maximum (i.e., 100% In). The Weibull model is suitable when asymmetric data, like plant fresh weight, define a response variable at a different rate than could be described using a log-logistic curve (Price et al. 2012). Selected models were chosen when they converged across the applicable dataset(s) and when the Shapiro-Wilks normality assumptions were met ($\alpha = 0.05$). For each species by metric modeled, lack-of-fit tests were performed to ensure the selected model was appropriate. Graphical outputs from the models used log-transformed values of tested florpyrauxifen-benzyl concentrations for each species response.

A Dunnett's test ($P < 0.05$) was performed in RStudio (R v. 4.0.3, R Foundation for Statistical Computing, Vienna, Austria) to establish the lowest observed effect concentration (LOEC) between the nontreated and treated plant dry biomass at the select florpyrauxifen-benzyl concentrations using the BASE and MULTCOMP packages (Horthorn et al. 2008; R Core Team 2022). An index comparing the estimated herbicide tolerance of invasive plants to the estimated herbicide susceptibility of the native plant (I/N ratio) was also calculated. The I/N ratio was defined as the estimated dry weight EC_{50} value of invasive plant species (Hydrocharitaceae; I) divided by the corresponding native species (*M. triphyllum*; N) dry weight EC_{50} value (EC_{50} invasive species/EC_{50} native species).

Results and Discussion

Nontreated reference plants exhibited shoot elongation and axillary branching during the 21-d static exposure. Nontreated plant biomass increased by 2.5 to 9.5 times that of the pretreatment biomass, which conforms to experiment validation standards of the OECD protocol (OECD 2014). The tested florpyrauxifen-benzyl concentrations evaluated in this study ranged 0.02% to 225% of the commonly recommended formulated product maximum use rate (48 µg L^{-1}) for submersed plant applications; therefore, plant control was compared directly using predicted EC_{50} values of shoot length, fresh weight, and dry weight metrics.

The native species, *M. triphyllum*, was the most sensitive plant evaluated in this study, with a dry weight EC_{50} value of 1.2 µg L^{-1} and LOEC of 0.4 µg L^{-1} (Table 2; Figure 1). Within 1 to 2 DAT, *M. triphyllum* exhibited auxin herbicide exposure symptoms, with epinasty appearing as the first sign of plant injury (data not shown). At the 2 wk after treatment (WAT) evaluation, plant symptoms had progressed to necrotic shoots with black nodes at concentrations >1.33 µg L^{-1}. Following plant harvest, *M. triphyllum* treated with >11.98 µg L^{-1} had <20% biomass remaining, and shoot lengths were reduced by 65% relative to the nontreated plants (Figure 1). These results confirm our hypothesis that *M. triphyllum* is highly sensitive to florpyrauxifen-benzyl, as the EC_{50} values in this study align well with previous small-scale herbicide-screening observations among other *Myriophyllum* spp. (Netherland and Richardson 2016; Richardson et al. 2016).

The most sensitive invasive species tested was *L. major*, which had an estimated dry weight EC_{50} value of 35.4 μg L^{-1} and LOEC of 35.96 μg L^{-1} (Table 2; Figure 1). Nevertheless, an EC_{50} for shoot length was not achieved (shoot inhibition $\mu < 50\%$). *Lagarosiphon major* injury from florpyrauxifen-benzyl was also rapid, with proximal bending and minor chlorosis observed within 1 to 3 DAT (data not shown). Leaf abscission from the apical shoots ensued ca. 7 DAT, when plants were gently agitated using forceps, with apical shoots fragmenting at concentrations >11.98 μg L^{-1} 2 WAT. Howell et al. (2021) noted a similar response to herbicide in an outdoor mesocosm study in which *L. major* exposed to florpyrauxifen-benzyl at 30 and 50 μg ai L^{-1} displayed proximal leaf abscission and stem fragmentation at 5 to 7 DAT. At harvest, *L. major* shoots treated with concentrations >66.58 μg L^{-1} reduced biomass more than 62% of the nontreated plants. Though sensitive, the calculated I/N ratio suggests *L. major* would require 31.3 times more herbicide to produce EC_{50} values similar to that of the native species, *M. triphyllum* (Table 2).

Elodea canadensis was not as sensitive to florpyrauxifen-benzyl as *L. major*, with fresh and dry weight EC_{50} value estimates greater than the highest concentration tested of 107.86 μg L^{-1}. Consequently, the LOEC for *E. canadensis* was also >107.86 μg L^{-1}. Shoot length inhibition did not meet the criteria for estimating an EC_{50} at any tested concentration (shoot inhibition $\mu < 50\%$) (Table 2; Figure 1). While response metrics suggest low sensitivity in this study, plant injury and growth abnormalities were present. Shoots became brittle, with necrotic tissue forming at the nodes with exposures to concentrations >11.98 µg L^{-1} at 2 to 3 WAT (data not shown). Conversely, a slight increase in axillary branching was noted at harvest among several plants treated at ≤11.98 µg L^{-1}. Based on the I/N ratio, *E. canadensis* would require >90 times more herbicide to produce an EC_{50} value comparable to that of *M. triphyllum* (Table 2). Similarly, Beets et al. (2019) noticed *E. canadensis* biomass was not affected when testing florpyrauxifen-benzyl at 3 to 27 µg ai L^{-1} in a 60-d concentration and exposure (CET) study.

Of the Hydrocharitaceae species evaluated, *E. densa* was the least sensitive, with EC_{50} and LOEC values not achieved with any test concentrations (Table 2). The trend in the data appeared linear with a shallow slope, indicating limited plant response to increased florpyrauxifen-benzyl concentrations during the 21-d exposure (Figure 1). Much like *E. canadensis*, apical portions of treated plants displayed auxin herbicide exposure characteristics, with slight epinasty, shoot twisting, and internode lengthening observed at 2 WAT at concentrations ≥35.96 µg L^{-1} (data not shown). However, these abnormal growth patterns did not appreciably reduce biomass or shoot lengths collected at harvest (Table 2). Howell et al. (2021) noted 80% *E. densa* visual control was not achieved until 7 WAT with florpyrauxifen-benzyl at 30 µg L^{-1} in outdoor mesocosm studies. Likewise, Haug et al. (2021) indicated *E. densa* had less shoot absorption and translocation than the Hydrocharitaceae species *H. verticillata* in a ^{14}C experiment applying 10 µg ai L^{-1} florpyrauxifen-benzyl during a 192-h exposure period. These previous studies suggest longer static exposure periods (e.g., >4 wk) may improve control of *E. densa*, as indicated by the low sensitivity shown in the present study, especially at lower concentrations (e.g., <36.96 µg L^{-1}). Consistent with this hypothesis, Madsen et al. (2021)

showed *E. densa* dry biomass was reduced by approximately 50% compared with the nontreated plants following a 10-wk static exposure to 50 µg ai L^{-1} florpyrauxifen-benzyl.

Elodea canadensis and *E. densa* proliferation in axillary branching and shoot development at the lower treatment concentrations (≤11.98 µg L^{-1}) further conveys the typical synthetic auxin properties of florpyrauxifen-benzyl, despite limited overall efficacy (personal observation). Hormesis, or augmented growth following sublethal herbicide concentrations, is characteristic of low-dose auxin injury (Belz and Duke 2014; Cedergreen et al. 2007; Jalal et al. 2021). Hormesis was noted in a previous study that documented a stimulated increase in yield for *E. densa* treated with the auxin herbicide, 2,4-D, applied at 1 to 11 mg ai L^{-1} (Peres et al. 2016). Similarly, Mudge et al. (2021) suggested potential hormesis occurred for *E. canadensis* in a 6-wk CET study when exposed to florpyrauxifen-benzyl at 3, 6, and 9 µg ai L^{-1}. While macrophyte hormesis literature is limited for florpyrauxifen-benzyl, findings from these previous auxin herbicide screenings closely align with the observations of *E. canadensis* and *E. densa* response to treatment in the present study. Further, these data denote the perceptive effective dose thresholding, which can occur among auxin herbicides, and the varying sensitivity found even within the same plant family (e.g., *L. major* dry weight EC_{50} was ~3-fold less than *E. canadensis* in the present study). Further research is required to specifically evaluate the lower florpyrauxifen-benzyl threshold concentrations and exposures that deter possible hormesis in common field application scenarios; notably in high water-exchange situations (e.g., flowing systems).

Though complexities of induced hormesis do exist with auxin herbicides (Belgers et al. 2007; Peres et al. 2017), submersed plant tolerance and sensitivity to synthetic auxins is well documented (Getsinger et al. 2003; Haug and Bellaud 2013; Hofstra and Clayton 2001; Parsons et al. 2001; Richardson et al. 2016; Sperry et al. 2021; Wersal et al. 2010). Unlike nonselective herbicides like diquat-dibromide, synthetic auxin herbicides, like 2,4-D and triclopyr largely act as selective compounds, which typically do not adversely affect monocotyledons compared with dicotyledon (broadleaf species) counterparts (Gettys et al. 2020; Madsen 2000). However, as part of the arylpicolinate class of auxins, florpyrauxifen-benzyl associates with a binding-site receptor atypical of common predecessor auxin classes (e.g., 2,4-D belongs within the phenoxy-carboxylate class) (Epp et al. 2016; Hoyerova et al. 2018; Lee et al. 2014). The mobility of florpyrauxifen-benzyl acid metabolites (Haug et al. 2021) and subsequent auxin derivatives within susceptible aquatic plants denotes the unique activity levels of this herbicide, as several selectivity phenomena were evident in the present study. For example, Hofstra and Clayton (2001) noted that *M. triphyllum* was not controlled in greenhouse trials using triclopyr at 0.25 to 2.5 mg L^{-1}. Yet rapid sensitivity and plant death was observed for *M. triphyllum* at very low florpyrauxifen-benzyl concentrations (≤1.2 µg ai L^{-1}). In the same trial, triclopyr did not provide adequate control of *L. major* in New Zealand (Hofstra and Clayton 2001), while *L. major* showed rapid sensitivity with no signs of recovery at florpyrauxifen-benzyl concentrations >35 µg ai L^{-1} in the present study.

While literature documenting submersed plant control with florpyrauxifen-benzyl is still developing at the international scale, results from the present study corroborate the findings of *Myriophyllum* spp. sensitivity among previous studies (Beets et al. 2019; Haug et al. 2021; Richardson et al. 2016). However, our findings do contradict those originally founded by Netherland and Richardson (2016), which indicated greater sensitivity for *E. canadensis* with EC_{50} values of 6.9 and 13.1 µg ai L^{-1}, as *E. canadensis* EC_{50} required more than twice the maximum labeled concentration of formulated herbicide in this 21-d study. Florpyrauxifen-benzyl degrades primarily through photolysis (1- to 2-d half-life), with secondary degradation occurring through hydrolysis with increasing pH (pH 7 to 9; 111- to 2-d half-life, respectively) (Heilman and Getsinger 2018; MDA 2018). Though unlikely, treatment pH in this study (μ = 8.2) could have nominally influenced herbicide activity on *E. canadensis*. A rapid conversion of florpyrauxifen-benzyl to the less-active parent acid under growth chamber conditions could have also reduced the observed herbicide activity on the more tolerant invasive plants, although this was not specifically tested for (Netherland and Richardson 2016; Richardson et al. 2016; Sperry et al. 2021). A more likely explanation for greater *E. canadensis* sensitivity in prior research is the potential for genotypic differences between naturalized plant populations in New Zealand versus the native range cohorts in North America. In New Zealand, *E. canadensis* accessions occur solely via clonal propagation, whereas viable seed production can occur within various regions in North America (Swearingen and Bargeron 2016). Past genetic comparisons were performed within established Hydrocharitaceae populations in New Zealand (Lambertini et al. 2010); however, this type of study typically focuses on species plasticity and invasion potential within invaded waterways rather than genetic parallels to the endemic plant populations. Further genetic screening comparing test species in the United States and New Zealand would allow for more relevant plant response comparisons for confirmation of this hypothesis.

In conclusion, this study indicates florpyrauxifen-benzyl would be a prospective candidate for *L. major* management, with further evaluation required to develop tactics that produce adequate control levels for *E. canadensis* and *E. densa*. Large-scale mesocosm trials would be beneficial in elucidating *E. canadensis* whole-plant response to validate plant tolerance metrics shown in this study, as previous outdoor mesocosm experiments showed more favorable results for *E. densa* control (Howell et al. 2021). Given the sensitivity of *M. triphyllum* compared with the Hydrocharitaceae tested, any targeted concentration used for invasive plant control in field scenarios would likely seriously injure native *Myriophyllum* spp. However, resource managers should note that native species, such as *M. triphyllum* and red pondweed [*Potamogeton cheesemanii* A. Benn.], generally produce large seedbanks that could support reestablishment following invasive plant eradication programs (Hofstra et al. 2018; de Winton and Clayton 1996; de Winton et al. 2000). As documented in previous studies evaluating florpyrauxifen-benzyl for invasive submersed plant control (Beets et al 2019; Richardson et al. 2016; Sperry et al. 2021), future research is needed to test additional native species' sensitivity for best management guidance. Similarly, future investigations should assess native and invasive species in mixed communities to show side-by-side confirmation of results in field-based plant management scenarios. While small-scale screenings can overestimate herbicide activity on plants (Richardson et al. 2016), this study exemplifies the benefits of alternative small-scale trials for quickly gauging submersed plant response to new herbicide chemistries and provides a foundation for future screening activity that could prove expedient for herbicide registration purposes.

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TABLES

Table 4-1. Herbicide concentrations used to evaluate species sensitivity to florpyrauxifen-benzyl in the growth chamber study.

Species	Family (division)	New Zealand plant status	Evaluated concentrations µg ai L^{-1}
Myriophyllum triphyllum	Haloragaceae (dicotyledon)	Native	0, 0.01, 0.04, 0.13, 0.4, 1.33, 4, 11.98, 35.96, 107.86
Lagarosiphon major	Hydrocharitaceae (monocotyledon)	Invasive	0, 0.13, 0.4, 1.33, 4, 11.98, 35.96, 66.58, 107.86
Elodea canadensis		Invasive	
Egeria densa		Invasive	

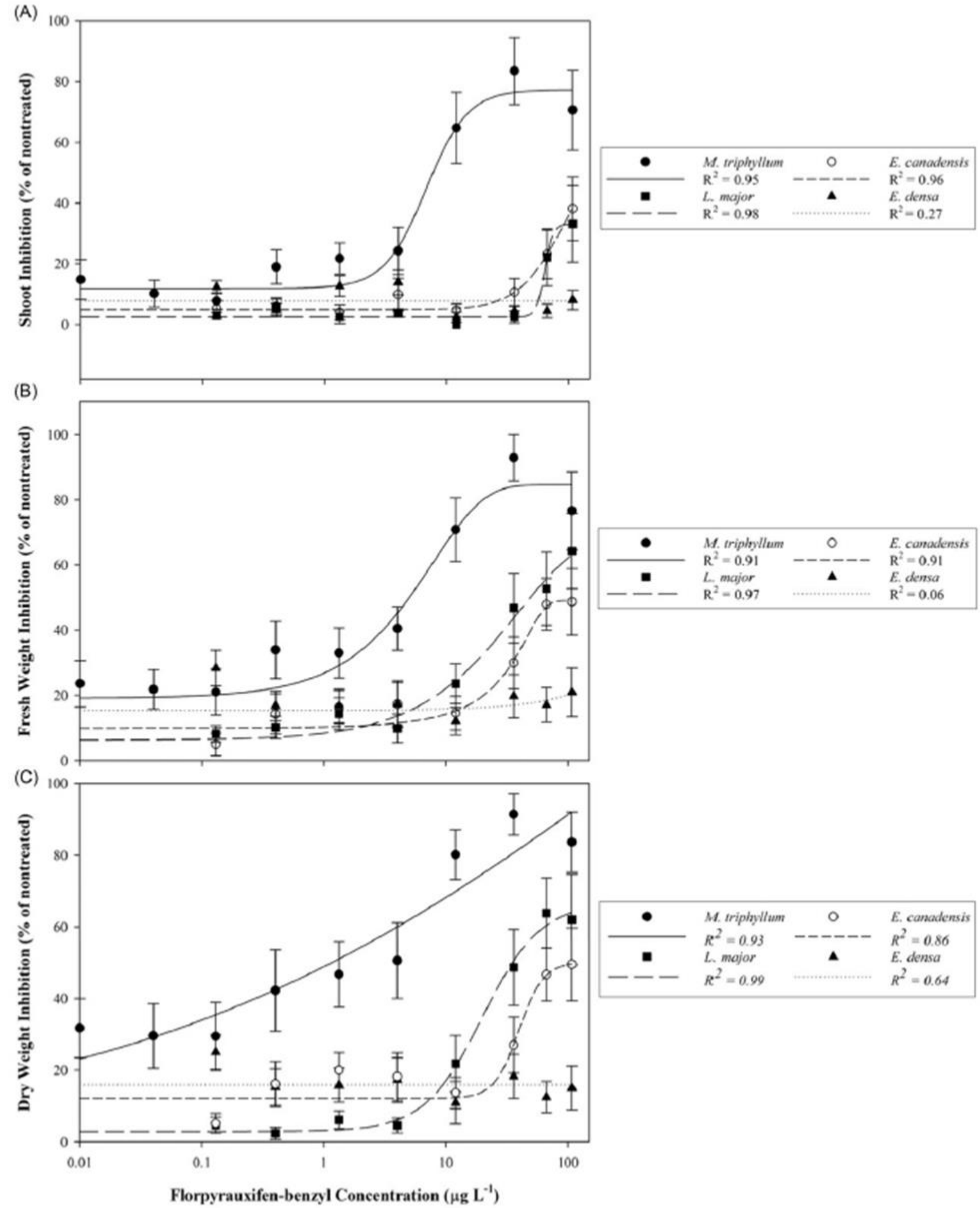
Table 4-2. Metrics of 50% effective concentrations (EC_{50}) derived from log-logistic four-parameter or Weibull four-parameter dose–response models following plant exposure to florpyrauxifen-benzyl at 0 to 107.86 μg ai L^{-1}, the lowest observed effect concentration (LOEC) calculated from Dunnett's test at the 0.05 significance level, and invasive-to-native plant tolerance index.

Species	EC_{50} florpyrauxifen-benzyl metrics[a]				I/N ratio[b]
	Shoot length inhibition	Fresh weight inhibition	Dry weight inhibition	Dry weight LOEC	
	μg ai L^{-1}				
Myriophyllum triphyllum	4.7	5.0	1.2	0.4	—
Lagarosiphon major	na	49.5	35.4	35.96	31.3
Elodea canadensis	na	> 107.86	> 107.86	> 107.87	93.8
Egeria densa	na	na	na	na	na

[a] EC_{50} values were not achieved (na) if species exhibited low inhibition ($\mu < 50\%$) for the metric tested.
[b] Estimated invasive species (Hydrocharitaceae; I) dry weight EC_{50} value divided by the native species (*M. triphyllum*; N) dry weight EC_{50} value.

FIGURES

Figure 4-1. Native (*Myriophyllum triphyllum*) and invasive (*Lagarosiphon major*, *Elodea canadensis,* and *Egeria densa*) plant responses following 21-d static exposure to tested florpyrauxifen-benzyl serial concentrations expressed as percent inhibition of the nontreated control: (A) shoot inhibition, (B) fresh weight inhibition, and (C) dry weight inhibition. Data points with standard error bars represent mean response of the metric evaluated. Herbicide concentration is provided on a $\log_{10}$ scale. Regression analyses implemented for plant shoot and dry weight inhibition correspond to the log-logistic four-parameter model equation: $Y = y_o + \{a/[1 + (x/x_{\mathrm{EC50}})^b]\}$, while fresh weight inhibition was modeled using the Weibull four-parameter equation: $Y = a \times \{1 - exp[-(x - x_{\mathrm{EC50}} + b + ln(2)^{1/c}))/b)^c]\}$.


(A)
(B)
(C)
Shoot Inhibition (% of nontreated)
Fresh Weight Inhibition (% of nontreated)
Dry Weight Inhibition (% of nontreated)
Florpyrauxifen-benzyl Concentration (μg L-1)
M. triphyllum
E. canadensis
L. major
E. densa
R2 = 0.95
R2 = 0.96
R2 = 0.98
R2 = 0.27
R2 = 0.91
R2 = 0.91
R2 = 0.97
R2 = 0.06
R2 = 0.93
R2 = 0.86
R2 = 0.99
R2 = 0.64

CHAPTER 5: Estimating standing biomass of exotic macrophytes using sUAS

Conference Proceeding Paper in SPIE

Howell AW, Richardson RJ (2019) Estimating standing biomass of exotic macrophytes using sUAS. Proc. SPIE 11008, Autonomous Air and Ground Sensing Systems for Agricultural Optimization and Phenotyping IV, 110080Z (14 May 2019). SPIE Defense + Commercial Sensing, 2019, Baltimore, Maryland, USA doi: 10.1117/12.2519199

Abstract

With the advent of small-unmanned aerial vehicles (sUAS), research scientists and plant managers are capable of obtaining unique, fast, and low-cost quantitative data, which delivers many repeatable survey options. Benefits of autonomous sUAS platforms include minimal training, reduced human safety concerns, and creation of graphic outputs, which may be readily viewed by any stakeholder who was not actively involved in the survey or management activity. Research conducted in the Wellington Region, New Zealand was used to evaluate consumer-grade sUAS technologies to map and estimate standing biomass of Manchurian Wild Rice (MWR) [*Zizania latifolia* (Griseb.) Hance ex F. Muell.], an exotic semi-aquatic grass which promotes flooding, and displacement of native flora and fauna. The goal of this research was to improve the speed and resolution of current survey strategies used to assess MWR among a lowland pasture site using unmanned systems and photogrammetry techniques. Image collection and data processing was conducted in a manner to provide a theoretic biomass estimation of remaining MWR following seasonal growth and herbicide applications. Post-processing methods and theories discussed attempt to identify and quantify MWR biomass using supervised imaging

analysis, plant height modeling, and biomass collected *in situ*. The use of unmanned systems to map, monitor, and manage MWR is encouraged for future applications.

Introduction

Aquatic invasive species (AIS), such as Manchurian Wild Rice (MWR) [*Zizania latifolia* (Griseb.) Hance ex F.Muell.], often have undesirable effects on native aquatic ecology and the associated local expenditures within invaded regions (Arnold 1959; Hofstra and Champion 2007; Santos et al. 2011; Gallardo et al. 2016). Generally, AIS like MWR reduce aquatic ecosystem biodiversity through resource competition (Madsen and Sand-Jensen 1991), dominance and displacement (Houlahan and Findlay 2004), and habitat disruption (Thum and Lennon 2009; Schultz and Dibble 2012). Specifically, the growth habits of MWR form impenetrable canopies that create waterlogged pasturelands and hazards to livestock (Figure 1). Although AIS threaten ecosystem processes (Wilcove et al. 1998), the expenditures associated with AIS are equally noteworthy as tall, perennial grass species like MWR commonly impede navigation, and obstruct canals and water intakes. To reduce the negative ecological and economic impacts associated with AIS like MWR, managers employ various schemes to protect and preserve native aquatic systems (cultural, mechanical, chemical, and monitoring tactics). One of the most effective methods to mitigate the spread of exotic plants is through early detection and rapid response measures (Westbrooks 2004), and regions which receive early detection minimize the financial and environmental costs associated with invasions (Rejmànek and Pitcairn 2002; Hestir et al. 2008; Thum and Lennon 2009; Lambert et al. 2010). However, when AIS incursions persist, such as the case with MWR in the Wellington Region, New Zealand, aquatic resource managers must integrate AIS surveillance and delimitation methods with subsequent control methods and monitoring.

It is well accepted that timely monitoring and mapping techniques are essential for evaluating native and exotic aquatic vegetation (Maceina et al. 1984; Hestir et al. 2008; Santos et al. 2011; Husson et al. 2016). Though many surveying techniques are well-established (Ackleson and Klemas 1987; Schneider et al. 2004; Gunn et al. 2010), *in situ* point-intercept sampling and 'search radius' protocols remain an industry standard for describing spatiotemporal trends in AIS presence, abundance, distribution, and richness. However, there are several hindrances with both methodologies, as assessments require a skilled workforce (correct species identification) and there is often subjectivity among surveyors, which can lower survey accuracy and efficiency. Likewise, these methods require considerable labor and time inputs, as the extent of waterway evaluations are highly correlated with the precision, spatial coverage, and time-length spent evaluating each sampling location. Therefore, researchers and plant managers seek to utilize recent advances in remote imaging and photogrammetric technologies, specifically small-unmanned aerial vehicles (sUAS), to minimize the expenditures associated with localized AIS surveys.

For the past several decades, optical sensors have aided in aquatic plant survey methods (Ackleson and Klemas 1987; Sawaya et al. 2003; Nelson et al. 2006; Hestir et al. 2008). Coupling remotely sensed data with computer-based programs, such as geographic information system (GIS) (e.g., ArcGIS; GRASS GIS; ENVI; RStudio), has further increased the performance of mapping techniques (Lehmann and Lachavanne 1997; Shaw 2005; Santos et al. 2016, Petrasova et al. 2017). Measurements of submersed and emergent vegetation have been successfully performed using true-color and multispectral imagery (Ackleson and Klemas 1987; Peñuelas et al. 1997; Belliss and Pairman 2009; Martin et al. 2010). However, the cost of repeated applications over large spatial scales often limits the use of many of the purchased satellite flights or airborne sensors. Multispectral open-source sensors, such as Sentinel-2 and Landsat 7 TM/ETM+, do provide the basic spectral ranges needed for accessing broad population estimates, but lack the spatial and temporal resolution (e.g. pixel size of 10-30 m^2 and ~5-15 days; respectively) to represent localized vegetation or differentiate amongst similar species. Most of the compact cameras used on sUAS provide superior spatial resolution and temporal determination (e.g. pixel size of 1–3 cm^2 and minutes-to-hours), with a fraction of the economic input of a single multispectral image from a commercial airborne or satellite sensor (Baluja et al. 2012; Turner et al. 2012; Candiago et al. 2015). Nevertheless, there are limitations with true-color and multispectral platforms for acquisition of aquatic vegetation. Plant physiological and environmental factors (e.g. turbidity, sun angle geometry, weather) can create complications when evaluating some aquatic plants, especially when observing canopies partially submersed in the water column. Still, implementing a sUAS could address the above limitations, while providing superior spatial resolution (<1-5 cm^2) with a user defined temporal resolution.

The objective of this research was to serve as a pilot program to provide a timely and systematic appraisal of MWR growth, objective population dynamics, and delineate post-treatment herbicide effects of the invasive semi-aquatic plant species located in the Wellington Region, New Zealand. Specifically, this study describes capturing aerial images of MWR at varying altitudes to develop maps and estimates of plant biomass using image classifications and *in situ* collected plant metrics. A protocol for comparing *in situ* collected biomass to plant height models derived from sUAS are described to combat the aforementioned surveying limitations. Findings from this study will be useful in supporting survey options and management tools, which would remove the subjectivity of biomass estimations in the field, while providing quantitative evidence of MWR stands to stakeholders.

Materials and Methods

Test Site: Wetland and low-lying pastureland (Wellington Region, North Island, New Zealand)

The study site was located near Waikanae Beach park (40°52′30″S 175°03′50″E, altitude: 2-5 m above mean sea level), 60 km north of Wellington City (Figure 2). This invaded area has been under MWR management regimes for the past 15 years. Herbicide application via helicopter or backpack sprayers occurs annually, and occasionally twice-annually, depending on the funding year with monitoring efforts following each successive management application period. Two locations (location 1:14 ha plot, and location 2: 5 ha plot; respectively) were flown at altitudes of 30, 61, and 120 m to identify the appropriate altitude needed to assess MWR stands. Destructive biomass sampling occurred among the first study location where standing biomass was harvested from three MWR density scales using 0.25 m^2 quadrats: (N=9; 3 replications: Density 1 [0-1 m], Density 2 [1-2 m], Density 3 [>2 m]). Biomass collections represented the minimum and maximum growth structures of MWR in the invaded site. Ground control points (GCPs) were distributed randomly across both study locations (Table 1; Figure 3). The GCPs were represented using latex party balloons inflated to a diameter of 18 cm and attached to emergent vegetation or fencing using monofilament fishing line (Figures 3 and 4). Each GCP position was measured using a handheld global positioning system (GPS, Garmin Rino 650, Australia/New Zealand) with 3 m horizontal and vertical position accuracy.

Biomass sampling

Destructive aboveground biomass sampling ensued separately amongst the sampling sites within the first study area (Table 2). Aboveground tissue (above soil or waterline) samples were cleaned of other plant materials and detritus. Harvested leaves representing each density level were bundled using bailers twin and measured for average leaf length prior to drying. To obtain above-ground dry biomass, each of the samples were dried to a constant dry-mass at 30 °C for 2 weeks, and each plant bundle weighed separately.

sUAS platform

In this study, a DJI Phantom 4 Advanced quadcopter was used to capture aerial photography of the study sites. The flight duration varied between 3–24 minutes based upon the altitude and flight plan. Due to battery and environmental conditions, flights were conducted over two days between the hours of 10:00 and 14:00 (18 April and 19 April). Pix4D Mapper and DroneDeploy tablet applications were used to develop and conduct each flight plan. Contingent on the flight application used, the DJI provided gimbal mounted RGB optical sensor (20 MP; FOV 84° 8.8 mm) maintained near-nadir and slightly oblique camera positions throughout the duration of each fight mission. During each flight, environmental conditions and sUAS locations were manually monitored and recorded. Captured imagery and associated metadata was automatically logged to an onboard 32 GB SD memory card.

Image pre- and post-processing

To produce an orthomosaic image and digital surface model (DSM) from each flight plan, Agisoft PhotoScan Pro photogrammetric structure from motion (SfM) software was utilized. The first process in creating a projected DSM and orthomosaic was to align the photos then assign GCPs to the imagery workspace. To successfully reference each GCP, at least three images containing each unique GCP was required. To help optimize the alignment process, GCPs were filtered by photo markers to use GCP .txt file coordinates for spatial error reduction. Prior to building dense clouds, the boundary box containing each region of interest was resized to encompass only the minimal extent of the model elements to reduce processing time and increase efficiency. For point cloud (Figure 5), mesh, and texture construction, a medium resolution batch process was used. Parameters for DSM and orthophoto creation were obtained from batch processing results. Data exports (.tiff) for further processing and reports (.pdf) were exported for further spatial error analysis.

Additional GIS processing occurred using ESRI's ArcGIS 10.4.1, QGIS 2.18, and GRASS GIS 7.4.0. Only orthophotos and DSMs from the first study location at 61 m were used to develop model parameters for supervised image analysis, plant heights, and point cloud differences among LiDAR (1 m) digital elevation model (DEM) and SfM results (Figure 6). The DSMs were resampled to represent the biomass sampling quadrat area (0.25 m^2). Biomass collection points were associated among each orthophoto to confirm well-known MWR stands for image classification training sample selection. Identified MWR stands from the classification techniques were then extracted from the resampled DSM to provide a raster dataset representative of MWR plant height estimates derived from the sUAS (Figure 7).

Statistical analysis and modeling

Regression analysis and biomass prediction algorithms utilized RStudio base, nonparametric, and ggplot2 packages (Hayfield and Racine 2008; Wickham 2009; R Core Team 2018). Employing ArcGIS 10.4.1 raster calculator, plant height raster datasets were imported into the MWR biomass regression, developed from *in situ* collected plant height estimates and biomass bundles, to acquire dry biomass estimates of the othrophoto scene.

Results and Discussion

Due to the complexity of plant community diversity among the test sites, and thus the requirement to classify individual MWR plant stands, only the 61 m flight altitude results for this study are presented to provide a proof-of-concept. Image capture parameters for the following data included a flying altitude of 61 AGL, which took 10 min to complete to cover 13.61 ha (N = 157 images collected).

Study site characteristics

While MWR was the only species of interest in this study, two other similarly structured species were present in the image scenes, which included *raupō* (Cattail; *Typha* spp.) and *harakeke* (New Zealand flax; [*Phormium tenax* (J.R.Forst. & G.Forst.)]). Each alike species (in terms of aerial discrimination) was heterogeneously distributed among the imaged portions of the landscape. This plant distribution pattern did not impede image correlation with MWR, as biomass samples collected *in situ* were selected among sites comprising <5% non-MWR species so not to skew the biomass prediction equation during analyses.

Plant height and biomass estimations

Although our plant presence classification methods likely overestimated the presence of MWR, note that identified MWR regions were co-referenced with flight logs procured during the previous year's helicopter application treatment. This allowed for a direct correlation between computer based image classifications of MWR and known MWR populations among the target sites identified by the aerial applicators.

Quadrat collection sites throughout the defined experimental area represented a wide range of plant heights, biomass, and MWR stand densities to account for growth variability (density height means = 143-276 cm; density weight means = 210-1812 g dm). Biomass collections varied less among Density level 1, than Density levels 2 or 3. Mean plant height to dry-mass ratios notably differed among each sampling density (Density 1: 2.26; Density 2: 6.96;

Density 3: 5.34), suggesting that plant height per unit area is an important factor to consider when describing expanses of low growth plant stands.

Comparing the *in situ* acquired plant heights and biomass provided a regression equation used for importing the MWR classified sUAS imagery (Figure 8). Biomass estimates showed strong agreement with MWR plant height and density estimates collected in-field ($r^2_{adj} = 0.61$). While variation among plant density levels are presented, our trends suggest a pattern of linearity, with the greatest deviance produced among plant stands 1.5-2.5 m tall. Employing the *in situ* trained regression equation with raster algebra and classification statistics, we estimate the MWR plant stands represented in location 1 (13.61 ha) at 985.79 kg dry mass (Figure 9). Though we recognize this estimate is likely location specific, and possibly altitude dependent if image classification is required, future-imaging missions over this location may desire to use or estimate as a baseline measurement to compare future estimates.

Survey methods and equipment

The Phantom 4 Advanced sUAS operated completely autonomously using a mission developed through PiX4D Mapper mobile application, which allowed for image capture repeatability and transfer of missions over time. Not only does this procedure reduce spatial coverage errors for future comparisons, but allows those unfamiliar with the equipment or study site to collect data without recreating the original flight plan. We also found no issue with camera image alignment amongst any of the imaging missions above 30 m (only 1 image went unaligned at the lowest altitude tested) during processing using this methodology.

We found using GCPs was critical when developing plant height estimates and fixing relative location errors. While the GCPs used in this study provided a quick solution for not having fixed GCPs in the landscape, we suggest future applications among semi-aquatic environments use static georeferenced GCPs if forthcoming flights are scheduled (eg. 6-8 GCPs per imaged site, with each GCP represented by a permanent pole with a distinguishable terminal target). One issue, which can arise using balloon GCPs, is if wind gusts are present the chance of the balloons deflating increases. Furthermore, while relative accuracy is increased using inflatable GCPs, the spatial precision can decrease due to windy conditions. We also make awareness that a precision GPS with fine resolution (5-10 cm vertical accuracy) should be implemented when placing GCPs. Nevertheless, our results show that relative accuracies can achieve biomass estimations of exotic macrophytes.

Future biomass estimation research

Among future studies, metrics of interest might include how patchy distributions of MWR in an orthomosaic scene accounts for biomass prediction success. For example, how does biomass calculation under scenarios of high plant diversity compare with monoculture stands? Likewise, what effect does plant morphology and phenology have on detection ability among sites with similarly structured species? Based on our findings, there remains a further need to determine how plant stand densities (number of plants per unit area and true spatial surface area) affects aerially derived biomass estimations.

Some example equipment and methods requiring further testing to overcome some of the known limitations include:

- Survey grade GPS equipment
- Fixed Ground Control Points
- sUAS with RTK/PPK capabilities

Conclusion

Mapping is crucial for risk assessment of plant invaders, quantifying ecosystem changes, and providing sound tactics for water resource conservation, management, and stewardship. This study defines additional toolsets and protocols, using photogrammetric and GIS strategies, to meet mapping demands. With the rapid development of off-the-shelf sUAS platforms, the potential exists to incorporate unmanned technologies into monitoring and mapping strategies for MWR control efforts. These tools may additionally be incorporated with traditional methods, such as ‘search and locate’ surveys, so that managers have the benefits of each technique. Implementing autonomous sUAS could provide an additional cost-effective and consumer available toolset, which is scalable and provides a reproducible method for MWR monitoring and management. Based on results, we suggest that our sUAS equipment and image capture strategies defined in this study are capable of providing greater than proof-of-concept evidence for MWR aerial biomass estimation. Not only does an aerial biomass assessment provide greater objectivity by the surveyor than manual binary estimates (e.g., presence/absence from ground or helicopter GPS tagging) but also allows for repeatable survey applications. This low-cost system is capable of providing parallel among many emergent aquatic species, systems, and environments. Likewise, this innovative research will prove useful in the invasive plant management industry as remote image collection and photogrammetric advancements progress. The data and information collected from sUAS surveys will contribute to the successful management, sustainability, and stewardship of agricultural and environmental resource systems in the future (Adam et al. 2010; Baluja et al. 2012; Vega et al. 2015). Estimating standing biomass of an exotic macrophyte species like MWR is crucial in monitoring successional growth and eradication program efforts. Findings from this study may be used for stakeholder reporting and management prescriptions.

Management Implications

Annually, the New Zealand biosecurity force and third-party researchers, conduct widespread surveillance to determine new infestations and prevalence of MWR plant stands. Traditional methods of this operation include manned helicopters and wading surveys using legacy or predicted infestation areas. This system requires a well-skilled workforce of many experienced professionals with extensive knowledge in piloting safety, significant familiarity with localized plant population dynamics, equipment upkeep, and is often difficult to repeat consistently on a multi-year program. The cost efficiency of this current regime could be increased if there were reductions to labor, time, and safety concern confronted with these surveys. Unmanned aerial technologies require minimal training, remove most human safety concerns, and provide deliverables, which may be viewed by any stakeholder within minutes to hours.

Estimates of MWR coverage, distribution, and biomass have been collected over the last decade in many areas within the North Island, NZ regions. These estimates have traditional been used to assess the vigor and impact MWR has had on a particular region for the fiscal or calendar year. Annually, active management has occurred following these plant survey assessments, which has included physical plant removal alongside herbicide treatment programs. Monitoring and assessing the temporal success or trends that follow these management regimes has become a costly and time intensive investment.

Outcomes from this study may also be suited to correlate legacy-data biomass estimates held by stakeholders. Imagery may be further subjected to a variety of other post-processing models to identify and quantify pre- and post-herbicide treatment effects using machine-learning, image classification (e.g., Canopeo mobile application), and geostatistical techniques. Waterway documentation and image capture intervals of vegetative growth could occur prior, during, and at set intervals after herbicide treatment (e.g., 2, 4, and 8 WAT) to provide a timeline of spectral or growth form response of MWR. For example, temporal and spatial persistence of MWR after treatment could follow visual ratings of percent control (efficacy appraisals of chlorosis and necrosis) from 0% (no injury) to 100% (complete desiccation) and automated imaging appraisals. Parallel treatment assessments of helicopter locations (monitored with GPS) versus backpack applications may follow an identical imagery protocol for comparison.

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TABLES

Table 5-1. Ground control points (GCP) of balloon location markers between both study sites.

GCP Identifier	Latitude	Longitude	Altitude (m)
MWR1	40.850757	175.049869	6.63
MWR2	40.851436	175.048517	6.41
MWR3	40.851974	175.049019	6.79
MWR4	40.851887	175.047712	5.50
MWR5	40.852345	175.047744	7.23
MWN6	40.852031	175.04782	8.43
MWR7	40.851189	175.048456	5.98
MWR8	40.851618	175.046948	4.68
MWR9	40.851054	175.046132	3.49
MWR10	40.851288	175.045775	3.64
MWR11	40.851414	175.046668	2.50
MWR12	40.851728	175.046696	2.69

Table 5-2. Destructive biomass harvest parameters of MWR in first study site.

Biomass Identifier	Density Level	Latitude	Longitude
01A	[0-1 m]	40.850935	175.04977
02A		40.851143	175.049544
03A		40.851156	175.049521
11B	[1-2 m]	40.850635	175.05058
12B		40.850606	175.050414
13B		40.850683	175.050261
21C	[>2 m]	40.852317	175.048487
22C		40.852389	175.048543
23C		40.852268	175.048321

FIGURES

Figure 5-1. Manchurian Wild Rice growth habit and invaded habitat examples. (A. Typical MWR growth structure with coarse leaves ranging 1-4 m in height/length.; B. Encroachment of drainage canal by invading MWR; C. Waterlogged pastureland with clumps of MWR spread throughout causing flooding issues). Often, livestock production is reduced due to the invasion of MWR into pastureland and can cause significant drainage issues in water catchment zones.

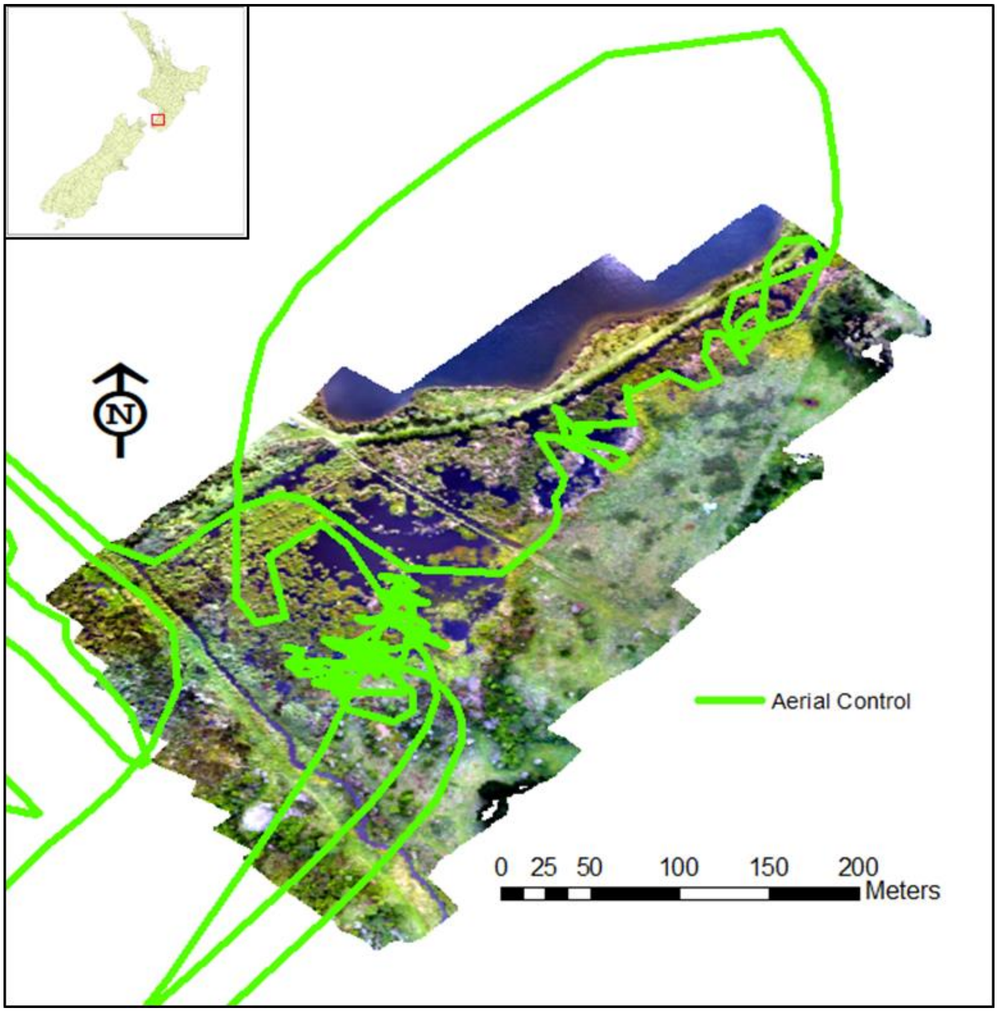

Figure 5-2. Representation of the study location 1 in Wellington, New Zealand with the first flight plan shown in the provided orthophoto (13.61 ha). The Wellington Region is located at the southernmost portion of New Zealand's North Island. This area is highly biologically productive and is currently impacted by several invasive macrophyte species. However, the area is also supporting populations of nationally threatened aquatic plants, local economies from agricultural operations, and native flora and fauna habitat that is often threatened due to dense stands of exotic vegetation. One of the most ecologically and economically detrimental weed species known to this area is MWR. Aerial control regimes are shown overlain in green on the othrophoto.

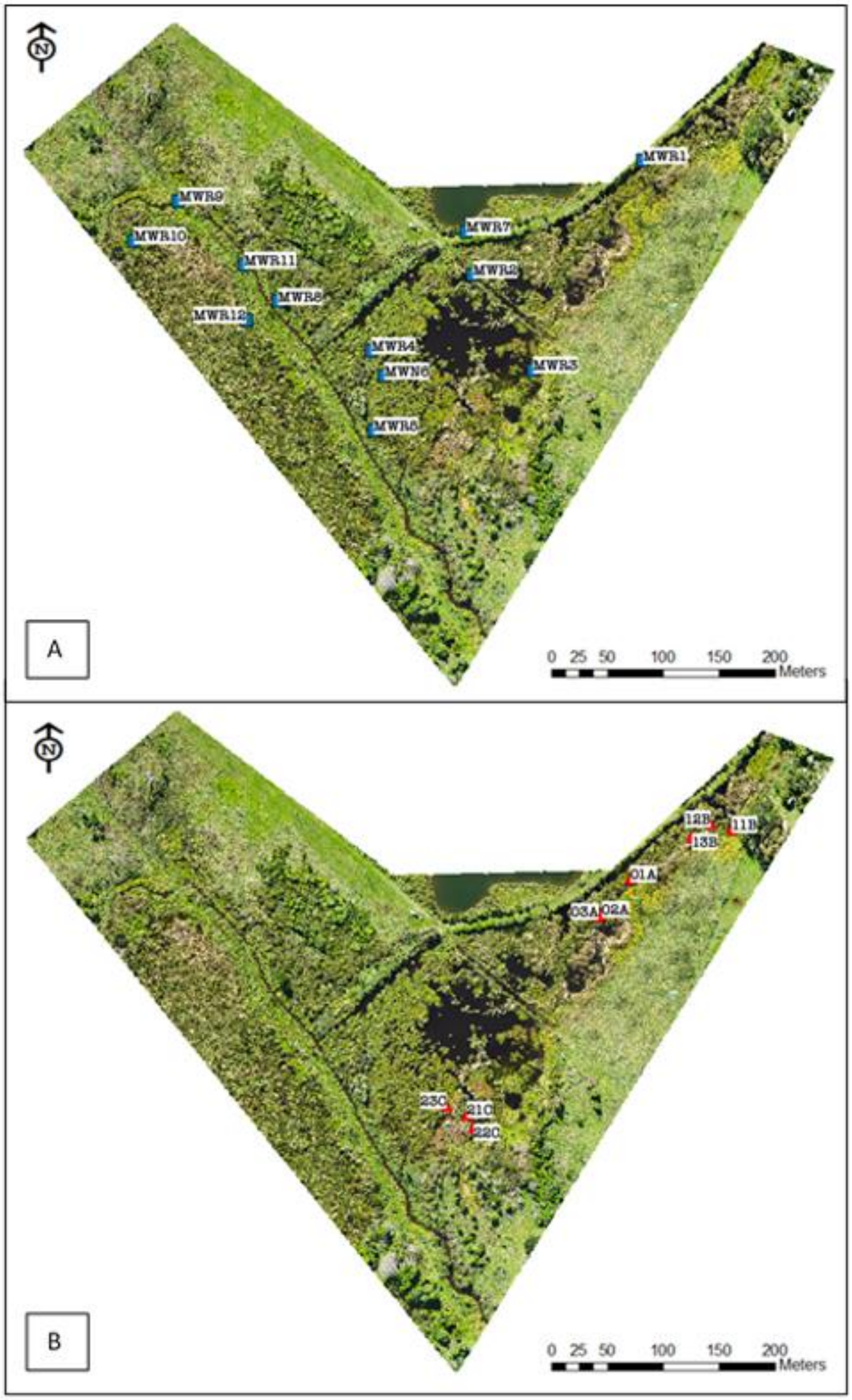

Figure 5-3. Maps showing an orthomosaic with respective GCP (A) and biomass harvest locations (B). Notably, the accuracy of the handheld GPS used for GCP reference was not ideal for use. However, the GCPs still provide a consistent baseline relative x-, y-, z-values among all altitudes flown.

Figure 5-4. Example of balloon used as a GCP. One of the confines with obtaining accurate and repeatable survey options among aquatic areas is the need for GCPs. Pink party balloons were used to meet the constraints of using GCPs in an aquatic environment. One downside to this method was the time it took to place each balloon, measure the GPS location, and fly the sUAS without the balloon popping. If this area were in need of continued monitoring, then placing a series of permanent poles having visible markings from a nadir perspective would be beneficial. This way, assessment time in the field would only be as long as the flight plan and permanent GCPs allow for a consistent baseline for temporal comparisons.

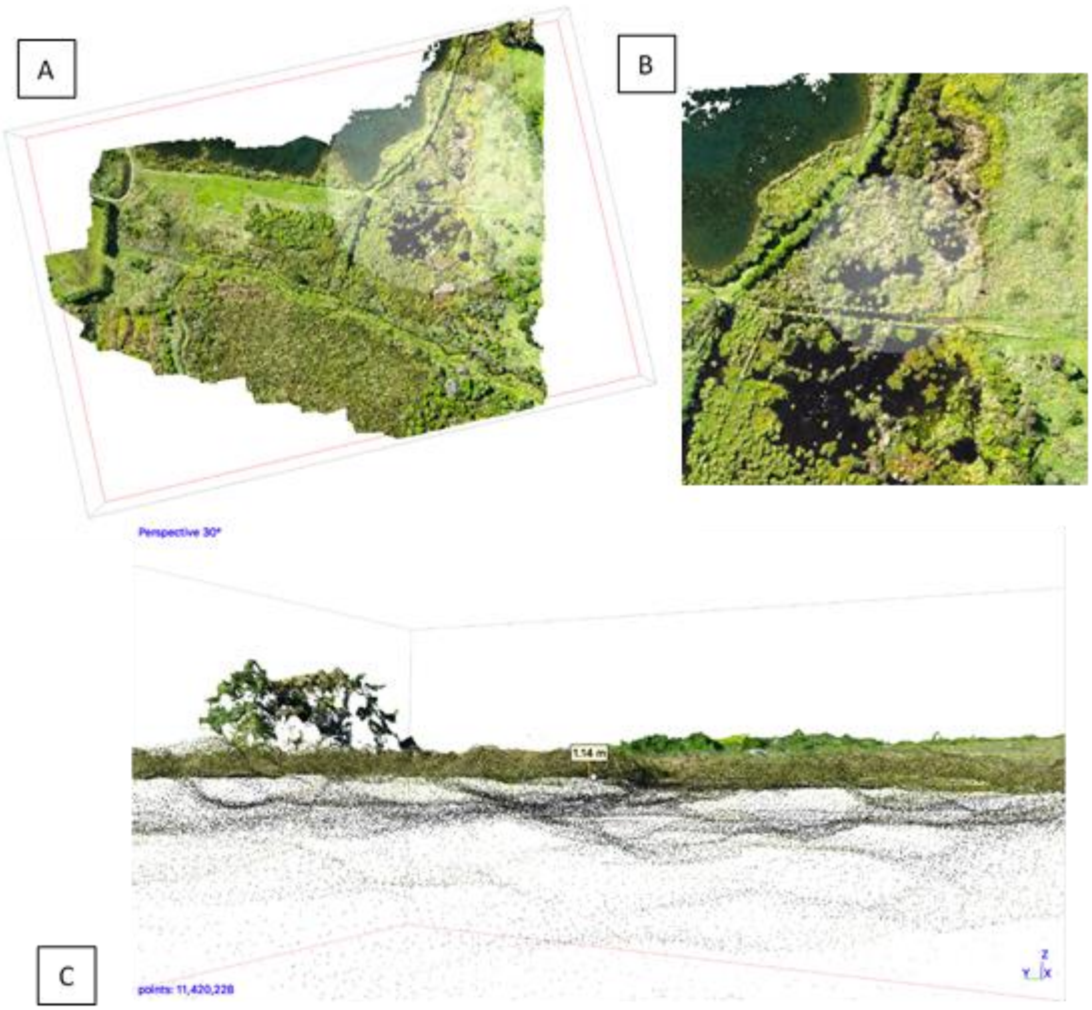

Figure 5-5. Dense point cloud views in Agisoft PhotoScan software of know MWR locations in the map (A. map overview; B. zoomed extent of overview; C. horizontal view of MWR stand with vertical measurement). Height measurements as shown may be validated against DSMs and helicopter treatment path within the map location.

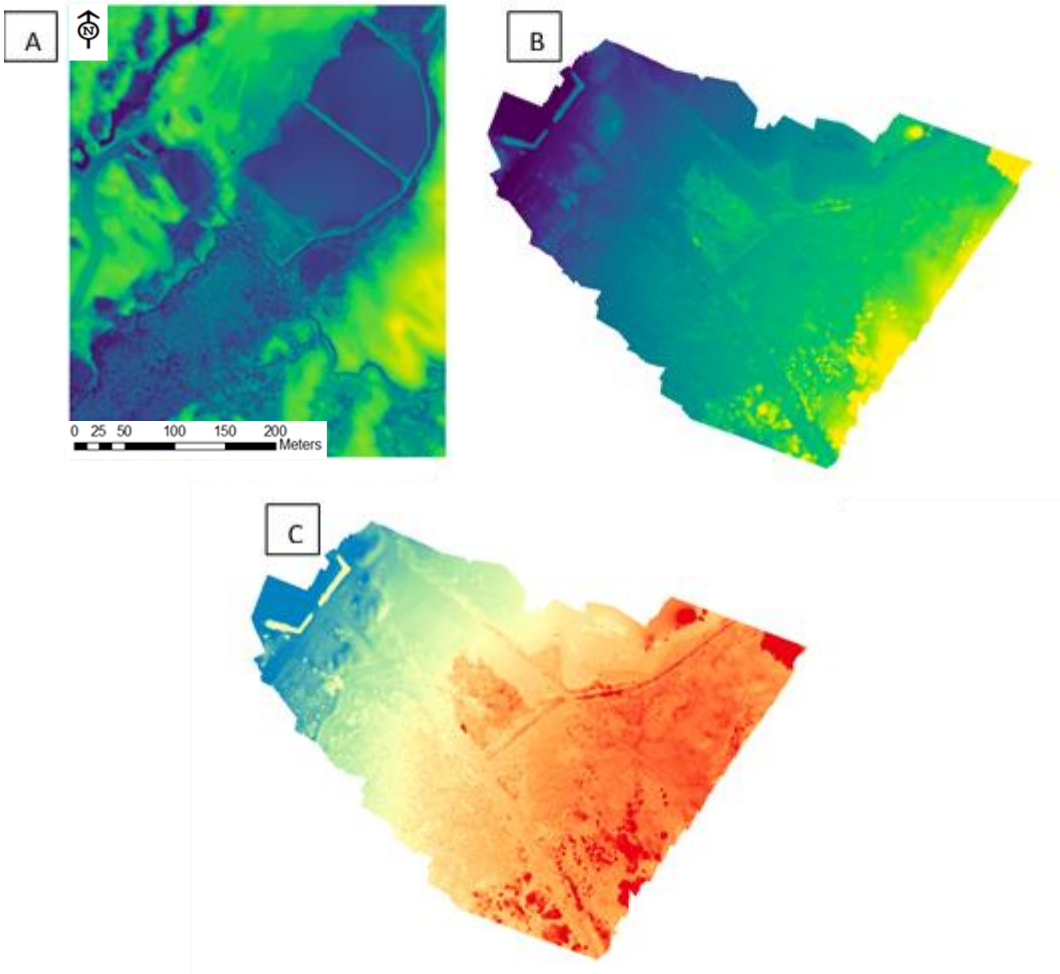

Figure 5-6. Example of the digital elevation and surface models of the first flight area at an altitude of 120 m AGL. Maps display: A. DEM 1 m Lidar data obtained through Land Information New Zealand (https://www.linz.govt.nz/data/linz-data/elevation-data); B. 12 cm pre-processed DSM done in Agisoft Photoscan software; C. Difference model created in GRASS GIS of A and B. Uncorrected elevation data for each map was A: (2.40 – 21.75 m ASL); B: (2.36 – 20.7 m ASL); and C: (0.75 – 14.5 m). All maps are set to the same spatial scale.

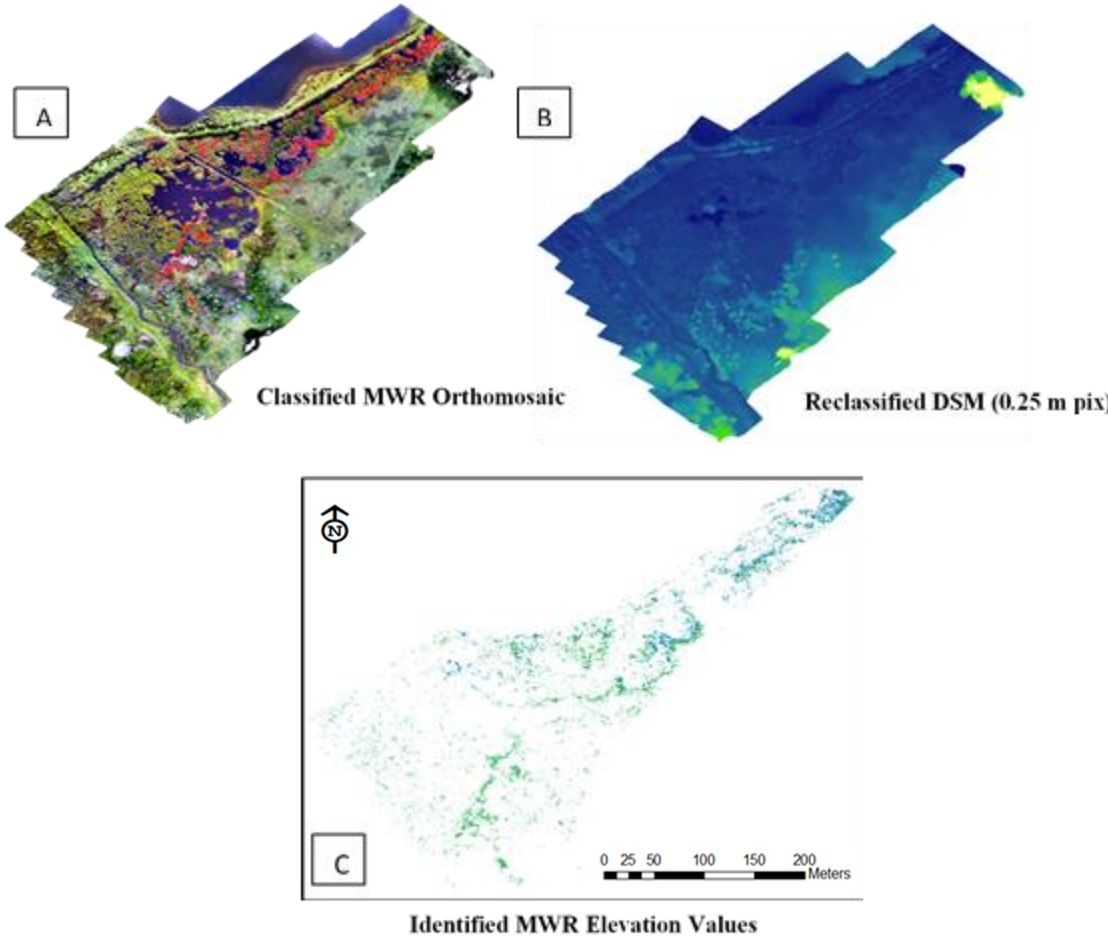

Figure 5-7. Supervised image classification mapping of MWR from the first study site at 61 m flying altitude. A). Orthophoto generated from this sUAS flight shows MWR classified as red. While the training data needs some manipulation, the classification represents known areas of MWR well. Classification used the biomass collection locations and previous helicopter application logs as an observational test of MWR representation. B). The reclassified DSM pixels represent the spatial coverage of each sampling density quadrat. C). Raster image representing MWR plant height values created by extracting the classified MWR orthophoto from the reclassified DSM. All maps are set to the same spatial scale.

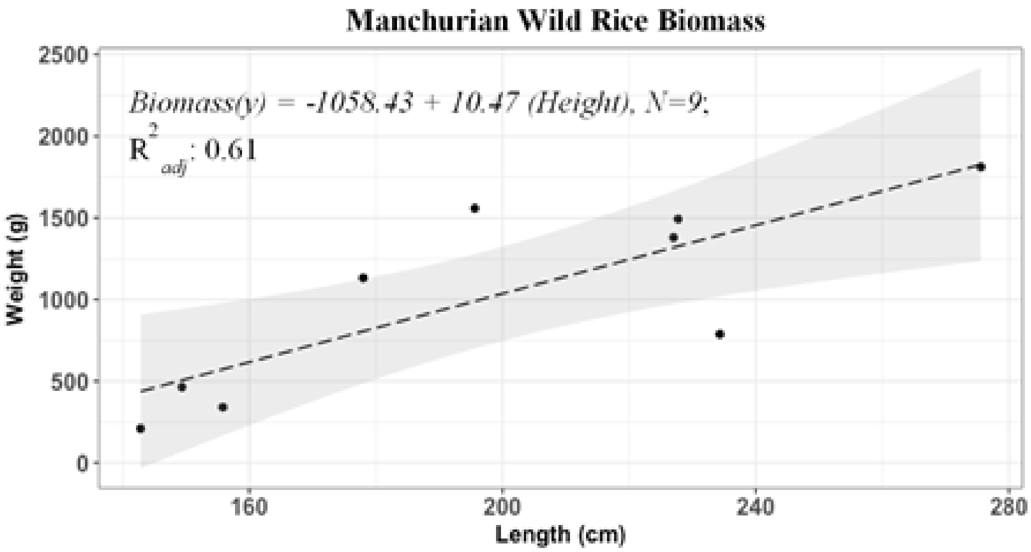

Figure 5-8. Regression model developed using *in situ* collected biomass and plant height estimates from each density 0.25 m^2 quadrat.

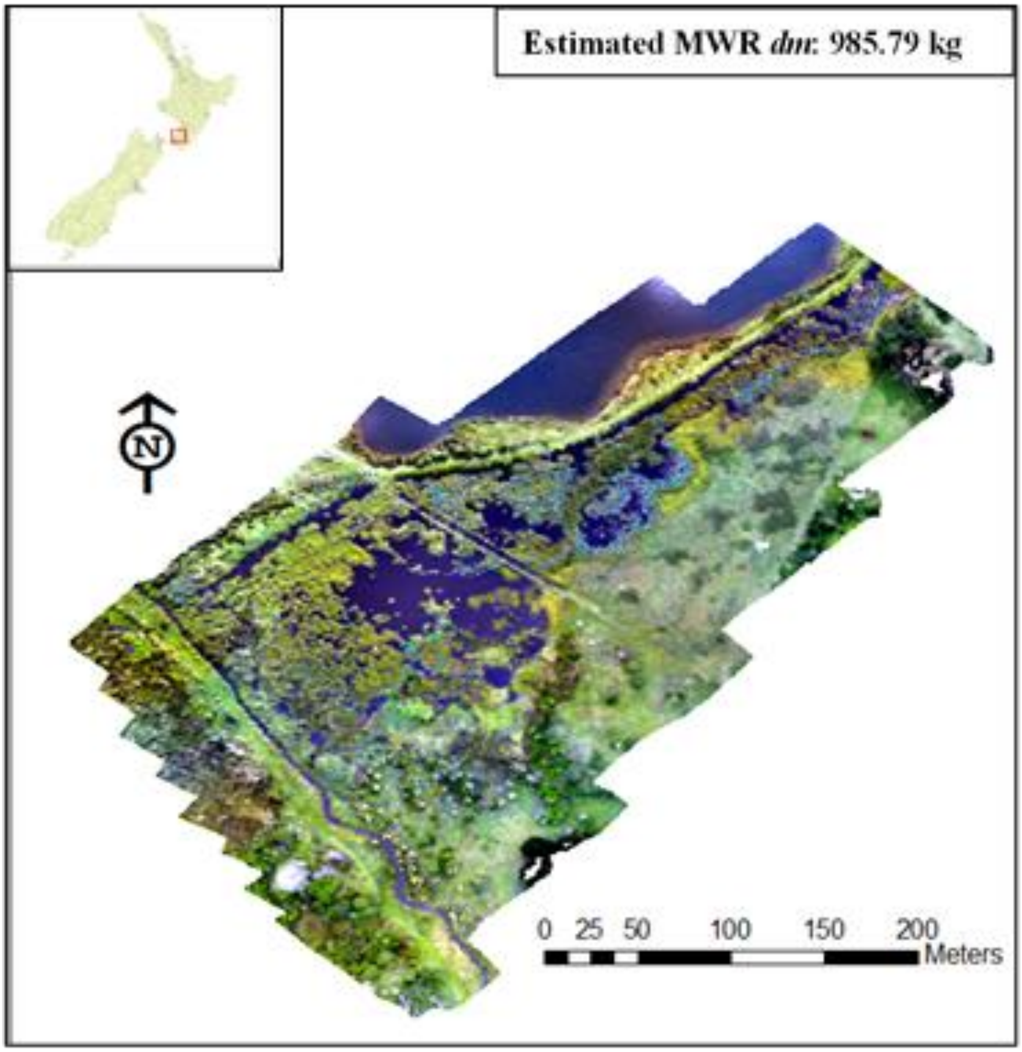

Figure 5-9. Map deliverable of MWR plant stands represented in location 1 (13.61 ha) estimated at 985.79 kg dry mass from aerially derived plant height estimates and the regression model. Plant stands are shown in the blue-green raster gradient.

CHAPTER 6: Low carrier volume herbicide trials and unoccupied aerial systems support eradication efforts of giant salvina (*Salvinia molesta*) in North Carolina

Formatted for Invasive Plant Science and Management

Abstract

Limited options are available for aquatic plant management, therefore expanding the current aquatic herbicide portfolio, reducing total spray volumes to increase operational efficiency, or remotely delivering herbicide using novel spray technologies could provide management benefits. However, research is limited on giant salvinia response to foliar herbicide applications at low carrier volumes (≤ 140 L ha^{-1}). Likewise, no data exists documenting herbicide efficacy from an unoccupied aerial application system (UAAS) for giant salvinia management. In 2021, three field trials evaluated registered aquatic and experimental herbicides at reduced carrier volumes (140 L ha^{-1}) within select sites of Gapway Swamp, NC. Results indicated the experimental herbicide, KFD-699-01 (424 g a.i. ha^{-1}), in combination with endothall dipotassium salt (2370 g a.e. ha^{-1}) improved plant control by 43% over KFD-699-01 (212 g a.i. ha^{-1}) applied alone, at 8 wk after treatment (8 WAT). Herbicide evaluations also showed diquat (3136 g a.i. ha^{-1}) alone, glyphosate (4539 g a.e. ha^{-1}) alone, and metsulfuron-methyl (42 g a.i. ha^{-1}) alone achieved 86 to 94% visual plant control at 8 WAT. Follow-by foliar applications of diquat, flumioxazin (210 g a.i. ha^{-1}), and carfentrazone (67 g a.i. ha^{-1}) 6 wk behind in-water fluridone exposures were not considered efficacious by 6 WAT. Carfentrazone applications made from a backpack sprayer displayed greater control than applications made with UAAS at 2 and 3 WAT; however, neither application strategy provided control at 8 WAT.

Due to the limited tank capacity of UAAS (≤ 10 L), a greenhouse trial investigated the efficacy of diquat, flumioxazin, and glyphosate at varying carrier volumes (23 to 935 L^{-1}). Greenhouse results indicated ultralow carrier volumes (23 L ha^{-1}) could increase UAAS operational performance, especially for diquat, which reduced plant biomass ≥ 98% at 4 WAT. Field validation of these results with UAAS deploying 23 L ha^{-1} carrier volumes is needed.

Introduction

Giant salvinia [*Salvinia molesta* (D. S. Mitchell)] is an invasive, free-floating aquatic fern that has progressively colonized wetland waterways throughout the southern United States over the past three decades (Johnson 1995; EDDMapS 2022). Native to Brazil (Fomo and Harley 1979), the federally noxious weed rapidly forms dense vegetation mats up to 1-m thick (Thomas and Room 1986), which can disrupt irrigation schedules, clog drainages, limit recreational opportunities, and result in human health concerns (McFarland et al. 2004). Ecologically, giant salvinia poses threat to habitat diversity by altering water chemistry (e.g., dissolved oxygen and pH) and restricting light availability to other aquatic biotas (McFarland et al. 2004; Owens et al. 2005). Once established, the floating fern is difficult to control due to the complexities of the waterways it occupies, its prolific growth rates (Kaufman and Kaufman 2007), and effective vegetative dispersal mechanisms (Nelson et al. 2001; Glomski and Mudge 2013).

In North Carolina, giant salvina was discovered in nine southeastern counties by the early 2000's (NCWRC 2015), but was considered eradicated in 2008 following rapid response measures with herbicides (W. Batten, personal comm.). The recent discovery (2020) of a new infestation in a cypress [*Taxodium distichum* (L.) Rich.] swamp system has again prompted rapid response techniques from local, state, and federal agencies (Rashash 2020; ERDC 2021). While aquatic plant management techniques like mechanical harvesting and biological control agents (e.g., salvinia weevil, [*Cyrtobagous salviniae* (Calder and Sands)]) can reduce giant salvinia density (Cook 1976, Cilliers 1991; Westbrooks 2010), herbicides or integrated management including herbicides are largely utilized for eradication efforts due to improved efficacy and selectivity (Nelson et al. 2001; McFarland et al. 2004).

Chemical control of giant salvinia in cypress swamps has been successful in Louisiana and Texas (Cozad et al. 2019); however, herbicide applications often challenge management due to limited watercraft accessibility and environmental constraints (e.g., trees provided refuge). Previous research has demonstrated subsurface (i.e., in-water) applications of the aquatic herbicides chelated copper, endothall dipotassium salt, fluridone, penoxsulam, and bispyribac-sodium have the potential to reduce floating plant biomass under greenhouse and field settings (Glomski et al. 2003; Mudge et al. 2012; Glomski and Mudge 2013; Mudge and Netherland 2020). However, these types of herbicide treatments can be cost-prohibitive or restricted due to localized constraints (e.g., irrigation restrictions) which can limit operational implementation for resource managers. Therefore, foliar-applied herbicides have largely remained the most common treatment technique for giant salvinia management.

The systemic herbicide, glyphosate, has persisted as one of the most effective foliar treatment options for managing giant salvinia (Nelson et al. 2007, Mudge et al. 2016). Still, the contact herbicides diquat, flumioxazin, and carfentrazone-ethyl (hereinafter, carfentrazone) have shown favorable results (Nelson et al. 2001; Glomski and Getsinger 2006; Richardson et al. 2008), and have been included as standalone treatments or as tank mix partners. Efficacy evaluations for 2,4-D at 0.75 to 3.0 kg a.e. ha^{-1} have also demonstrated 92 to 95% giant salvinia control in greenhouse settings (Diatloff et al 1979); however, low herbicide efficacy was reported in Brazil when 2,4-D was applied at 1.34 kg a.e. ha^{-1} to giant salvinia in a reservoir

(Martins et al. 2002). In the United States, 2,4-D is not readily utilized targeted giant salvinia control, but it does provide excellent control of the free-floating plant, water hyacinth [*Eichhornia crassipes* (Mart.) Solms] (Enloe et al. 2022), which commonly associates with giant salvinia in mixed stands. In recent studies, the herbicide metsulfuron-methyl at rates ≤ 42 g a.i. ha^{-1} provided ≥ 98% control when foliar applied (Sartain and Mudge 2018; Prevost et al. 2021). However, this herbicide can only be applied under the Federal Insecticide, Fungicide, and Rodenticide Act Special Local Need (SLN) 24(c) label in Texas, Louisiana, and South Carolina (Mudge 2020). While several foliar applied herbicides have been trialed and utilized to successfully combat giant salvinia (Nelson et al. 2007; Richardson et al. 2008; Glomski and Mudge 2013; Mudge et al. 2016; Sartain and Mudge 2019; Prevost et al. 2021), there remains an interest to expand the currently available aquatic herbicide portfolio while using lower carrier volumes (e.g., 140 L ha^{-1}) to increase operational efficiency and performance.

Typically, aquatic herbicides are delivered at total spray volumes between 468 to 1870 L water ha^{-1} (Nelson et al. 2007; Haller 2020). While this foliar application strategy has been successful with numerous herbicides and plant targets (Sperry and Ferrell 2021); there are associated hindrances that make “high” spray volume applications undesirable for current management tactics. Research has established carrier volume directly affects spray deposition, herbicide activity, and ultimately aquatic plant control (Van et al. 1986; Willard et al. 1998; Moreira et al. 1999; Nelson et al. 2007; Sperry and Ferrell 2021; Sperry et al. 2022). Limitations of high carrier volumes (e.g., 935 L ha^{-1}) include reduced spray retention at low floating plant densities, thus resulting in spray loss to the water column (Mudge et al. 2021), and reduced herbicide concentration per spray droplet (Knoche 1994). In general, herbicide applications made with lower carrier volumes are preferred because of time savings when filling spray tanks

(Nelson et al. 2007), public perception (Sperry and Ferrell 2021), increased spray retention on plant targets (Sperry et al. 2022), and the increased performance of some herbicides on floating plant species (Van et al. 1986; Nelson et al. 2007; Sperry and Ferrell 2021).

Traditional spray application techniques made with hand wands often requires applicators to navigate hazardous environmental conditions (e.g., submersed objects and stumps) to achieve adequate spray coverage for plant control. Inaccessible regions within a waterbody can also completely restrict foliar applications due to obstruction (e.g., trees; plant floatons) and limited water depth (e.g., too shallow for watercraft), making spray attempts futile. Over the past decade, unoccupied aerial application systems (UAAS) have gained popularity in terrestrial settings to remotely deliver herbicides among complex environments that limit access for ground-based operations (Göktoğan et al. 2010; Xue et al. 2016; Lan et al. 2017; Wang et al. 2019). Aquatic herbicide applicators have expressed the desire to integrate UAAS in spray operations to overcome inherent environmental complexities in aquatics and potentially decrease exposure to herbicides during treatment. However, commercially available UAAS typically have tank volumes of $\leq$ 10 L to conform to Federal Aviation Administration regulations (i.e., FAA 14 CFR Part 107). Existing application strategies limit UAAS operational use and further signify the importance of reevaluating carrier volumes for foliar spray tasks. To date, literature regarding UAAS practices among aquatic site applications remains limited; however, positive results have occurred when implementing UAAS for site-specific weed management over conventional broadcast herbicide applications in terrestrial sites (Hunter et al. 2020). While studies do exist comparing the efficacy and efficiency of UAAS to standard backpack applications in terrestrial settings (Hunter et al. 2020; Martin et al. 2020; Gertsis and Karampekos 2021), none have directly correlated herbicide efficacy holding carrier volume constant. There remains a clear need to investigate the performance of herbicides delivered from UAAS to traditional ground based applications made at low carrier volumes for aquatic weed management.

Although spray application techniques in terrestrial settings generally outpace weed management strategies in aquatics, there is an opportunity to adopt similar spray technology and techniques to enhance current floating plant management strategies. As weed science advances evolve with time, it is important to evaluate novel foliar spray methods to increase herbicide efficacy while increasing spray application efficiency. We hypothesize the reduction of carrier volume will achieve comparable results to current management tactics and thereby provide an additional application strategy for management. The objectives of this research are to: (1) evaluate the efficacy of aquatic and experimental herbicides at reduced carrier volumes for giant salvinia eradication efforts in NC; (2) document the performance of carfentrazone when applied with an UAAS versus foliar backpack applications; and (3) determine if reducing carrier volumes for diquat, flumioxazin, or glyphosate treatments could improve UAAS efficiency while maintaining expected efficacy.

Materials and Methods

Field Trials

Three outdoor trials were conducted within select sites of Gapway Swamp (Columbus Co., NC; 34.21°N, 78.94°W) to evaluate the efficacy of herbicides against tertiary growth giant salvina during the summer of 2021. Two trials occurred within a local farm pond (0.61 ha) directly adjacent to the main swamp system, and one trial occurred at a nearby beaver pond (0.24 ha) connected via culvert to the swamp. Prior to herbicide applications, floating quadrats (0.79 m^2) constructed of polyvinyl chloride (PVC) pipe and floatation foam were deployed to serve as reference plots for the selected herbicide treatments. Floating quadrats were secured in place using metal conduit anchors. Trials were initiated the 13th, 20th, and 28th of July and were monitored weekly over the 8-wk study period (trials 1, 2, and 3, respectively). Ambient environmental conditions at the time of the herbicide applications consisted of partly sunny skies with air temperatures of 28 C (± 0.7 SD), and wind speeds not exceeding 9.7 km hr^{-1}. At the time of herbicide treatment, plots were 100% covered with giant salvinia. Reference plots had pretreatment biomass values of 328.0 ± 58.5 g dry weight.

Trial one included varying rates of two experimental products, KFD-699-01 and KFD-393-01, and the registered aquatic herbicides 2,4-D amine (WSSA group 4; synthetic auxin) and endothall dipotassium salt (WSSA group 31; protein phosphatase inhibitor). Trial two evaluated the herbicides carfentrazone (WSSA group 14; protoporphyrinogen oxidase inhibitor), diquat (WSSA group 22; photosystem-I diversion), flumioxazin (WSSA group 14; protoporphyrinogen oxidase inhibitor), glyphosate (WSSA group 9; EPSP synthase inhibitor), metsulfuron-methyl (WSSA group 2; acetolactate synthase inhibitor), and penoxsulam (WSSA group 2; acetolactate synthase inhibitor). Trial three also included the herbicides carfentrazone, diquat, and flumioxazin; however, these applications were made as a follow-by application following an in-water treatment of fluridone (WSSA group 12; carotenoid biosynthesis inhibitor) that had occurred 6 weeks prior to trial initiation. A complete list of the herbicides used in these trials is included in Table 1. All herbicide treatments included a nonionic surfactant (Induce®, Helena Agri-Enterprises, Collierville, TN, USA) at 0.25% v/v. For each trial, a nontreated control was included to compare plant vigor without herbicide. All treatments followed a randomized block design and included four replicates. Foliar applications were applied from a boat using a CO_2 pressurized backpack sprayer with a handheld boom fitted with two XR110015-VP nozzles (TeeJet® nozzles, Spraying Systems Co., Wheaton, IL, USA) calibrated to deliver an application volume of 140 L ha^{-1}. Applications were made approx. 0.4 m above the plant canopy and when wind speeds were < 3.2 kph, to avoid spray drift onto adjacent plots. At both of the trial locations, an unoccupied aerial application system (UAAS) was deployed to compare the efficacy of carfentrazone backpack foliar applications to low-altitude (3 m above ground level; AGL) aerial carfentrazone foliar applications. Aerial applications occurred with a DJI Agras MG-1 octocopter (Da-Jiang Innovations, Shenzhen, China) consisting of four AIXR11002-VP (TeeJet® nozzles, Spraying Systems Co., Wheaton, IL, USA) nozzles calibrated to deliver 140 L ha^{-1}. For all trials, aerial imagery was collected with a DJI Phantom 4 Advanced (Da-Jiang Innovations, Shenzhen, China) small unoccupied aerial system (sUAS) at 9 and 61 m AGL to evaluate initial giant salvinia vigor, and plant injury or regrowth following herbicide applications since ground-based assessments were not feasible due to inaccessibility and concern of plot disturbance. Treatments were compared using visual estimations from aerial imagery to determine plant control (0 to 100%; no plant control to complete necrosis) among each evaluation. Due to weather prohibiting aerial image collection, evaluations could not be completed at 7 weeks after treatment (WAT) for trial one, 6 WAT for trial two, and 5 or 7 WAT for trial three. Additionally, fractional green canopy cover (FGCC) was calculated from collected aerial imagery in ImageJ software (National Institutes of Health, Bethesda, MD, USA) following similar methods as described by Ali et al. (2013), to provide a quantitative measure of the remaining viable plant material 8 WAT. This binary image processing procedure utilizes hue, saturation, and brightness color thresholding parameters, calibrated to the nontreated reference plots, to separate green plant material (pixels) from the non-green background (i.e., water and necrotic plant tissues) to calculate the percentage of green pixels. Following image collection, plots were destructively harvested 8 WAT and plants were force air dried for 72 hrs at 60 C, then recorded as dry biomass. All data were subjected to ANOVA and means were separated using Fisher's Protected LSD ($P \leq 0.05$) in RStudio (version 4.0.3; RCore Team 2020) using the packages: 'agricolae' (de Mendiburu 2020) and 'dplyr' (Wickham et al. 2021). Statistical comparisons of plant control by application technique between backpack and foliar treatments used Student's t-test ($\alpha = 0.05$) in RStudio. Linear regression analysis was performed in SigmaPlot 14.0 software (SigmaPlot 14.0.3.192, Systat Software Inc., Point Richmond, CA, USA) to compare the agreement between calculated FGCC percentages and visual control estimates at each trial's conclusion.

Greenhouse Trial

Two small-scale mesocosm trials were conducted in May through June 2022 at the North Carolina State University Aquatic Weed Control Labs (Raleigh, NC; 35.789°N, 78.694°W), to evaluate the influence of carrier volume on herbicide efficacy for giant salvinia control. Giant salvinia was field collected 4 April 2022 from the farm pond noted in field evaluation trials and cultured in 121 L vessels for two weeks in the greenhouse. Plants were then placed into each 4.5 L treatment mesocosms to achieve ca. 50% coverage. Mesocosms were filled with municipal tap water (pH 8.42) amended with 0.16 g of water-soluble fertilizer (24-8-16, Miracle-Gro® All Purpose Plant Food, Scotts Company, Marysville, OH, USA). Additional tap water was added to the mesocosms as loss occurred and fertilizer was reapplied biweekly throughout the 4-wk trial duration.

Herbicide applications ensued ca. 10 days following transfer to the treatment mesocosm when plants completely covered (100% coverage) the water surface with tertiary growth. Single rates of diquat (3136 g a.i. ha^{-1}), flumioxazin (210 g a.i. ha^{-1}), and glyphosate (4539 g a.e. ha^{-1}) were evaluated as foliar treatments at carrier volumes of 23, 140, and 935 L^{-1} (Table 1). A nonionic surfactant (Induce®, Helena Agri-Enterprises, Collierville, TN, USA) was included in all herbicide treatments at 0.25% v/v. A nontreated control was also included to compare plant response in the absence of herbicide. Treatments were applied using a controlled droplet application (CDA) sprayer (Herbi Handheld Sprayer, Micron Group, Bromyard, Herefordshire, UK) or a CO_2 pressurized backpack sprayer with a handheld boom consisting of a single XR11002-VP or XR11008-VP nozzle (23, 140, and 935 L^{-1}, respectively; TeeJet® nozzles, Spraying Systems Co., Wheaton, IL, USA). Foliar treatments were repeated in time and followed a randomized block design with four replicates. At treatment application, water pH was 5.7 (SD ± 0.15) and temperature was 25.3 C (± 1.98 SD). Giant salvinia response to treatment was evaluated weekly using visual estimations of plant control (0 to 100%; no plant control to complete necrosis) to determine the effect of carrier volume with evaluated herbicides. Nadir view digital images of the experimental units were collected (approx. 25 cm above the water surface) at each evaluation time point. Imagery collected 4 WAT was used to calculate FGCC to provide a quantitative measure of remaining viable plant material at trial conclusion. All viable giant salvinia was harvested 4 WAT and dried at 70 C for 48 hrs, then recorded as dry biomass. Nontreated reference plant biomass increased by two-fold over the 4-wk trial duration, indicating conditions were adequate for plant growth throughout experimentation. No significant interaction occurred between trial runs ($\alpha = 0.05$), therefore data from both runs were combined ($N = 8$). Using similar procedures as described for the field trials, data were subjected to ANOVA and means were separated using Fisher's Protected LSD ($P \leq 0.05$). Linear regression analysis was performed to compare the agreement between calculated FGCC percentages and visual control estimates.

Results and Discussion

Field Trials

Three field trials were conducted to evaluate the effectiveness of registered aquatic and experimental herbicides to support giant salvinia eradication efforts in Gapway Swamp, NC. Currently, low-carrier volume foliar application strategies are not commonplace in aquatics. However, aquatic resources managers could benefit from reduced spray volumes and interest exists to deploy UASS for herbicide applications. Nevertheless, limited demonstrations exist providing efficacy demonstrations between traditional ground-based foliar application techniques and UAAS herbicide applications. The aim of this research was to investigate the influence of herbicide selection and application technique for giant salvinia control when foliar applied.

Trial 1. Evaluation of experimental herbicides alone and in combination.

Within 1 WAT, all treatment plots showed varying levels of herbicide injury (29 to 96% visual control), which included chlorotic and necrotic fronds (Table 2). Initial visual control estimates indicated KFD-699-01 in combination with endothall or 2,4-D provided the greatest injury to treated giant salvina, having 96 and 71% visual control, respectively (Table 2). Previous studies have shown endothall alone or as a tank mix partner in foliar spray solutions provides rapid injury symptoms to giant salvinia within days to 2 WAT (Nelson et al. 2001, Mudge et al. 2016, Mudge and Netherland 2020), and supports the additive injury benefits shown in the present trial when endothall was included with KFD-699-01 as a tank mix partner. Across all treatments, visual control levels peaked 3 to 4 WAT with no difference between KFD-699-01 single or combination treatments (85 to 94% visual control). Plant injury from combinations of KFD-699-01 with KFD-393-01 progressed slower than the combinations of KFD-699-01 with either endothall or 2,4-D; however, by the 5 and 6 WAT evaluations, there were no differences between the highest rate of KFD-699-01 alone and KFD-699-01 with either rate of KFD-393-01, endothall, or 2,4-D. KFD-699-01 alone at 212 g a.i. ha^{-1} provided the least visual control during these later evaluations. At the trial conclusion (8 WAT), all treatments showed varying levels of regrowth. However, due to a high degree of variability in regrowth by 8 WAT, only KFD-699-01 in combination with endothall provided significant improvement in visual control (43% improved control) over KFD-699-01 applied alone at the lowest rate. Computer aided estimates of FGCC conveyed results similar to visual control estimates at 8 WAT, with KFD-699-01 in combination with endothall providing the least green canopy cover (31% less FGCC than the nontreated reference plots) and therefore the greatest plant control. Dry weight data showed a similar response as the visual and calculated image analysis metrics. Reduction in biomass was greatest with KFD-699-01 in combination with endothall; however, all other treatments did not differ from one another (Table 2).

Trial 2. Evaluation of commonly applied foliar herbicides alone and in combination.

At 1 WAT, all foliar herbicide applications resulted in chlorotic and necrotic fronds (data not shown), with visual control estimates ranging 82 to 93% (Table 3). Visual control continued to increase for all treatments 3 WAT, with varying levels of plant recovery observed 4 WAT among all treatment plots except for those treated with glyphosate or metsulfuron-methyl at 42 g a.i. ha^{-1}. Carfentrazone alone showed the highest recovery at this 4 WAT evaluation. At 5 WAT, carfentrazone + penoxsulam, diquat, flumioxazin, glyphosate, and both rates of metsulfuron-methyl provided 91 to 99% control (Table 3). A significant rate response ($P > 0.05$) was observed 7 and 8 WAT for metsulfuron-methyl, as plots treated at 42 g a.i. ha^{-1} improved visual control 78 to 80 percentage points greater than plots treated with metsulfuron-methyl at 21 g a.i. ha^{-1} (7 and 8 WAT, respectively). Sartain and Mudge (2018) indicated metsulfuron-methyl at 21 and 42 g a.i. ha^{-1} provided >98% control in a 12 week mesocosm study which, contradicts the difference in metsulfuron rate response presented in the current trial. However, previous research has shown that mesocosm results do not necessarily align with field assessments due to environmental variability (Netherland and Getsinger 2018). This could explain the significant rate response difference for metsulfuron-methyl in the present trial not observed in previous mesocosm trials. By 8 WAT, foliar treatments of carfentrazone, flumioxazin + glyphosate, and metsulfuron-methyl at 21 g a.i. ha^{-1} provided the least control (≤ 6% visual control) (Table 3). While not specifically tested for, we suspect antagonism occurred when flumioxazin and glyphosate were tank mixed, as flumioxazin alone and glyphosate alone provide significantly improved giant salvinia control (47 and 91 percentage points more visual control, respectively). However, previous research in Louisiana does contradict the hypothesis of flumioxazin + glyphosate antagonism which, showed these herbicides in combination provided >98% giant salvina control 7 to 8 WAT (Mudge et al. 2016; Mudge and Sartain 2018). While all treatments displayed giant salvinia recovery 8 WAT, plots treated with diquat alone, glyphosate alone, and metsulfuron-methyl alone at 42 g a.i. ha^{-1} did achieve 87, 94, and 86% visual plant control, respectively. Further, there was no difference 8 WAT in visual control estimates for diquat, glyphosate, and metsulfuron-methyl at 42 g a.i. ha^{-1} (Table 3).

Imagery analysis of FGCC closely supported the visual control estimates 8 WAT, with diquat, glyphosate, and metsulfuron-methyl at 42 g a.i. ha^{-1} providing the least green canopy cover (45, 43, and 41% less FGCC than the nontreated reference plots, respectively) and therefore the greatest control (Table 3). It should be noted the FGCC calculations for carfentrazone + penoxsulam, diquat, glyphosate, and metsulfuron-methyl at 42 g a.i. ha^{-1} were not different according to ANOVA ($P = 0.05$), which does differ from visual control evaluations at trial conclusion 8 WAT (Table 3). Dry weight data did not provide separation between treatments; further, no treatments differed from the nontreated reference biomass (Table 3). This response is likely due in part to the tertiary growth present at time of herbicide application. Previous studies have documented limited plant control following foliar applied herbicides to multiple plant layers due to difficulty in penetration of herbicide through the entire plant canopy (Mudge et al. 2016, Sartain and Mudge 2018). While contact herbicides like flumioxazin provide rapid injury symptoms initially, greenhouse and field studies have indicated giant salvinia recovery when applied alone (Richardson et al. 2008, Sartain and Mudge 2019). Additionally, plant biomass may not be the most appropriate metric to use when describing foliar applications made in small field plot scenarios, as dead plant material can remain floating with viable giant salvina contributing to the recorded biomass and reducing the ability to separate treatment responses. The results from regression analysis show a strong linear trend when FGCC image calculations act as a surrogate to visual control observations for gauging plant response to treatment ($R^2 = 0.77$) (Figure 1). Similar image analysis techniques are encouraged to monitor giant salvinia response to herbicide in future studies and plant management scenarios when plant biomass limits the ability to separate treatment responses.

Giant salvinia treated with the UAAS provided 80% visual control 1 WAT (Table 3). However, UAAS treatments began recovering by 2 WAT, with carfentrazone backpack applications showing improved plant control over UAAS 2 and 3 WAT (15 and 21% greater visual control, respectively). Nevertheless, there was no difference between carfentrazone application methods beyond the 4 WAT evaluation ($\alpha = 0.05$), and plants had fully recovered 8 WAT regardless of the treatment method (Table 3). The lack of control at trial conclusion is not surprising based on previous evaluations of carfentrazone that indicated retreatment might be necessary beyond 4 WAT to control remaining viable plant tissues leading to regrowth (Glomski and Getsinger 2006). Ramsdale and Messersmith (2001) evaluated the effects of adjuvant type on carfentrazone efficacy in terrestrial settings and showed 9 to 12% improved control of flax (*Linum usitatissimum* L.) and oilseed sunflower (*Helianthus annuus* L.) when a methylated vegetable oil (MVO) was included in the spray solution in place of NIS. Since giant salvinia is guarded by trichromes on the surface of plant fronds which reduces spray penetration and thus limiting herbicide effectiveness (McFarland et al. 2004), the inclusion of a more appropriate spray adjuvant (i.e., MVO at a higher % v/v) may have improved plant control over the inclusion of NIS at 0.25 % v/v in the present trial. Future evaluations should investigate the variability between ground and aerial applications of additional contact herbicides with varying spray adjuvants and the influence on giant salvinia control.

Trial 3. Evaluation of select contact herbicides as a follow-by application following in-water fluridone exposures.

Previous research suggests a follow up “bump” application of fluridone is often required 6 to 8 weeks after initial treatment to maintain suitable in-water herbicide concentrations to control established giant salvinia populations (Mudge et al. 2012). While single sub-surface fluridone treatments of 10 to 20 µg L^{-1} often severely injures giant salvinia (Mudge et al. 2012), regrowth occurs with frequency in well-establish populations (personal observation) and managers have suggested the desire to spot treat areas using foliar applied herbicides to control remaining plants. Likewise, the application of foliar herbicide treatments is generally more economical for control of targeted areas of regrowth than subsurface fluridone applications. This trial aimed to evaluate the effectiveness of foliar follow-by applications using the contact herbicides carfentrazone, diquat, and flumioxazin, which are commonly deployed for giant salvinia management (Casey Moorer, personal comm.).

Giant salvinia was partially bleached and chlorotic (data not shown) following an in-water application of fluridone 6 weeks prior to the foliar treatment trial initiation. Follow-by foliar applications of carfentrazone, diquat, and flumioxazin overtop fluridone treated plants resulted in rapid plant injury, with necrotic fronds appearing within 1 WAT, whereas nontreated reference plots displayed signs of recovery (healthy green fronds) following the previous fluridone treatment (i.e., 7 weeks following initial fluridone exposure, data not shown). Although visual control for all herbicides peaked at 97 to 99% 2 WAT, observations of giant salvinia recovery did occur across all treatment plots by 3 WAT (Table 4). At both the 2 and 3 WAT evaluations, carfentrazone applications made with the backpack technique displayed greater control than applications made via the UAAS; which, mimics trial two results using these spray techniques (Tables 3 and 4). However, no difference was observed between carfentrazone application methods at any other evaluation time point ($\alpha = 0.05$). Due to the fact carrier volume was held constant in these trials, we speculate that other factors such differences in spray droplet size and distribution (droplets per unit area) between the backpack and UAAS influenced the performance of the contact herbicide, carfentrazone (data not shown). Giant salvinia control was not considered efficacious for management by 6 WAT (35 to 43% visual control), and complete plant recovery was observed for all foliar treatments by the trial's conclusion 8 WAT (Table 4). Results from the quantitative measurements of FGCC and plant dry weights further support visual control estimates, with no difference detected between treated and nontreated plants at the eight-week harvest (Table 4).

Floating plant control frequently relies on foliar herbicide application strategies during early invasion scenarios and when targeting persistent or escape plants following active management. However, results from the present trial do not support the use of a single foliar application of the contact herbicides carfentrazone, diquat, or flumioxazin as a surrogate to maintaining, or increasing, giant salvina control when fluridone concentrations are in a lag phase (below lethal concentrations). While all foliar treatments provided excellent visual control (88 to 99%) within 2 WAT (Table 4), initial treatment efficacy was not synonymous with long-term effectiveness. Further, plant recovery following treatment occurred more rapidly than previously documented (Nelson et al. 2001, Glomski and Getsinger 2006, Richardson et al. 2008, Mudge et al. 2012, Mudge and Sartain 2018, Sartain and Mudge 2019). The rapidness of plant regrowth among all foliar treatments suggests mat thickness may have contributed to decreased herbicide performance, as giant salvinia mats were 7 to 10 cm thick at trail initiation. Another likely explanation for reduced herbicide performance was the decomposed and injured plant material present from the fluridone application, which could have shielded newly formed giant salvinia fronds from the foliar spray; thus, new growth was not exposed to herbicide. Previous research supports this hypothesis, as Sartain and Mudge (2019) attributed the presence of necrotic plant tissue to reducing the efficacy of diquat and flumioxazin when foliar applied to giant salvinia. Wersal and Madsen (2010) also observed a similar shielding response when common salvinia [*Salvinia minima* (Baker)] was sprayed with diquat at 130.8 g a.i. ha^{-1} to surface plant material which, allowed plants underneath the mat surface to recolonize. Since contact herbicides are not actively translocated within the plant phloem (Shaner 2014), the fast-acting nature of carfentrazone, diquat, and flumioxazin treatments evaluated in the present trial may have also limited the longevity of plant control (Sartain and Mudge 2019). There remains the need to evaluate the performance of additional slower-acting foliar herbicides, like glyphosate or penoxsulam, as follow-by treatments following in-water fluridone exposure and the influence of giant salvinia growth stage at time of the follow-by application (i.e., primary, secondary, or tertiary stage).

Greenhouse Trial

Due to mechanical and efficiency constraints, UAAS foliar treatments for giant salvinia requires the use of lower than standard carrier volumes. To ensure sufficient control with this application method, evaluations of carrier volume influence on herbicide efficacy ensued using results of likely herbicide candidates from field trials two and three. For the herbicides evaluated by carrier volume, rates remained constant with the field evaluations to provide a direct comparison of results.

All diquat treated plants displayed rapid injury symptoms, with necrotic fronds appearing within 1 day after treatment (DAT) (data not shown). At the 1 WAT evaluation, giant salvinia control due to diquat treatment was greatest at the 935 and 140 L ha^{-1} carrier volumes (99 and 96% visual control, respectively) (Table 5). Visual control ratings of plants treated at the 23 L ha^{-1} were different and less pronounced than the higher carrier volumes 1 to 3 WAT. Plant injury increased with time for all carrier volumes, with complete plant control (i.e., no remaining viable plant material) occurring 3 WAT for the two highest carrier volumes evaluated. Despite initial differences in visual control, by 4 WAT, no differences in plant control were detected for diquat regardless of carrier volume. Calculated metrics of FGCC and dry weight closely support the visual estimates of plant response to treatments, as only the 23 L ha^{-1} diquat applications had viable plant material remaining. Results from regression analysis further confirm the strong predictive relationship between FGCC image calculations and visual control observations for gauging plant response to treatment (R^2 = 0.79) (Figure 1). These data demonstrate the utility of diquat foliar applications for giant salvinia control across a broad range of carrier volumes and provide data supporting UAAS deployment since all spray applications reduced plant biomass ≥ 98%.

Similar to diquat, injury symptoms from all flumioxazin treatments were quick to emerge (Table 5). Yellowing of fronds was observed on 30 to 90% of plant material within 1 to 2 DAT, with some plants sinking at the 935 L ha^{-1} carrier volume by 3 DAT (data not shown). No difference was detected 1 to 2 WAT across carrier volumes for flumioxazin, however visual control differences did occur by 3 WAT, where the 935 L ha^{-1} carrier volume provided 9% improved control over 23 L ha^{-1} applications. By trial conclusion at 4 WAT, some plant recovery was apparent across all carrier volumes evaluated which, is not uncommon with single applications of flumioxazin on giant salvina (Sartain and Mudge 2019). Still, plants treated at the 935 and 140 L ha^{-1} carrier volumes provided the greatest visual control (91 and 87%, respectively) and differed from the lowest volume tested 4 WAT (23 L ha^{-1} provided <75% visual control). Calculated FGCC at plant harvest conveyed comparable separation of carrier volumes to the visual control estimates. All flumioxazin treatments reduced giant salvina biomass 72 to 88% (Table 5); however, increasing carrier volume from 23 L ha^{-1} to either 140 or 935 L ha^{-1} significantly enhanced biomass reduction. While the lowest carrier volume of flumioxazin was not as effective as the mid- and high volumes tested, plant control at 23 L ha^{-1} was significant, and would likely be an effective UAAS option if retreatment were to occur within 3 to 4 WAT as a follow-by application to limit regrowth, or target escaped plants.

As anticipated, injury due to glyphosate applications was more limited as compared to those made with the contact herbicides, diquat and flumioxazin. Plants treated with glyphosate typically show a phytotoxic response within 4 to 7 DAT (Shaner 2014), which supports initial injury response observations in the present trial. Giant salvinia displayed chlorosis and browning of frond margins by 2 to 3 DAT on 10 to 30% of plant tissues irrespective of carrier volume (data not shown). At the 1 to 3 WAT evaluations (Table 5), plants treated at the 935 L ha^{-1} carrier volume displayed significantly greater control (80%) than those treated at the lower application volumes (18 and 49% less control at 140 and 23 L ha^{-1}, respectively). Nevertheless, plant injury progressed with time for all treatments. By 4 WAT, the 140 and 935 L ha^{-1} carrier volumes performed similarly; however, when carrier volume was reduced to 23 L ha^{-1} visual control also declined by 26 to 34 percentage points. In previous research on water hyacinth (*Eichhornia crassipes* (Mart.) Solms), glyphosate efficacy significantly increased by 61% when applied at 187 L ha^{-1} compared with applications made at 935 L ha^{-1} (Sperry and Ferrell 2021). However, the present trial did not demonstrate improved control between glyphosate evaluated at 140 and 935 L ha^{-1} application volumes and several biotic and abiotic factors could help explain these differences. Influences of plant architecture (e.g., erect vs. prostrate), spray quality and retention (e.g., droplet size and distribution, and plant capture of spray solution), and leaf-surface morphology (i.e., pubescent vs. glabrous leaf surfaces; giant salvinia and water hyacinth, respectively) can greatly influence the performance of herbicide efficacy on floating plant species (Mudge et al. 2021, Sperry and Ferrell 2021, Sperry et al. 2022). A discrepancy of carrier volume performance using visual estimations and FGCC image analyses did occur at harvest. However, these metrics still suggested plants treated at 935 L ha^{-1} provided the greatest control. Differences between these assessment methods may be attributed to the presence of injured green plant material 4 WAT (e.g., fronds that contain ≤ 25% green tissues) since plant viability is not accounted for when conducting FGCC analytics. Nevertheless, dry weight calculations closely support the findings from visual control ratings at harvest, indicating glyphosate reduced giant salvinia biomass by 65, 77, and 80% of the nontreated control (23, 140, and 935 L ha^{-1}, respectively). Additional research assessing appropriate spray adjuvants, treatment rates, and specific spray deposition requirements to improve glyphosate's effectiveness at the lowest carrier volume (23 L ha^{-1}) for UAAS spray operations is needed to improve control.

This research provides the first published data that describes carrier volume influence on the efficacy of diquat, flumioxazin, or glyphosate directed at UAAS operations. Findings from the greenhouse evaluation suggest managers wishing to deploy UAAS for chemical management can achieve high levels of efficacy at 23 L ha^{-1} application volumes, though future field validation should occur to confirm results.

In conclusion, data from both field and greenhouse trials revealed low carrier volume foliar applications ($\leq$ 140 L ha^{-1}) can provide an effective alternative to the more commonly delivered spray volumes of 468 to 1870 L ha^{-1} for floating plant control (Nelson et al. 2007, Haller 2020). Results from field trials confirmed select experimental and registered aquatic foliar herbicide treatments show promise for controlling tertiary growth of giant salvinia up to 8 WAT. These data provide value for future giant salvinia eradication efforts in North Carolina and management of additional invasion sites throughout the United States. The inclusion of aerial image capture at each evaluation further enhanced separation of plant response to treatment. Image analytics previously described provide rapid quantitative assessment of plant phytotoxicity and future use of sUAS true-color imagery as an alternative evaluation technique to gauge treatment success is encouraged. Data from the greenhouse trial also supports the use of ultralow carrier volumes (23 L ha^{-1}) for UAAS application methods, though field verification of this method is required to confirm results. Future research should continue identifying low-volume application strategies with other herbicides and tank-mix partners, and evaluate UAAS directed in-water application techniques with appropriate herbicides for giant salvinia management.

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TABLES

Table 6-1. Herbicides evaluated in the three field trials and one greenhouse trial examining the control of giant salvinia under low-carrier volume applications.

Common Name	Trade Name	Manufacturer	Trial
2,4-D amine	Weedar® 64	NuFarm, Alsip, IL, USA	Field 1
Carfentrazone	Stingray®	SePRO, Carmel, IN, USA	Field 2, 3
Diquat	Reward®	Syngenta, Greensboro, NC, USA	Field 2, 3/Greenhouse
Endothall dipotassium salt	Aquathol® K	UPL, King of Prussia, PA, USA	Field 1
Flumioxazin	Clipper® SC	NuFarm, Alsip, IL, USA	Field 2, 3/Greenhouse
Glyphosate	Rodeo®	Corteva, Indianapolis, IN, USA	Field 2/Greenhouse
KFD-393-01	Experimental	UPL, King of Prussia, PA, USA	Field 1
KFD-699-01	Experimental	UPL, King of Prussia, PA, USA	Field 1
Metsulfuron-methyl	MSM 60®	Alligare, Opelika, AL, USA	Field 2
Penoxsulam	Galleon® SC	SePRO, Carmel, IN, USA	Field 2

Table 6-2. Visual percent control, fractional green canopy cover, and dry weights of giant salvinia following foliar herbicide applications in trial one.

Herbicide[d]	Rate[e]	Weeks After Treatment[a,b,c] 1	2	3	4	5	6	7	8	FGCC[f]	Dry Weight
	g a.i. ha^{-1}	%								%	g
KFD-699-01 + Endothall dipotassium salt	424 + 2370	96 a	96 a	96 a	94 a	94 a	94 a	--	78 a	20 a	148.7 a
KFD-699-01 + 2,4-D amine	424 + 2129	71 ab	64 a	96 a	94 a	94 a	92 a	--	73 ab	31 abc	208.1 ab
KFD-699-01 + KFD-393-01	424 + 208	21 de	69 bc	93 ab	93 a	91 a	91 a	--	66 ab	29 ab	285.4 bc
KFD-699-01 + KFD-393-01	424 + 104	53 bc	79 b	79 ab	90 a	85 ab	83 ab	--	46 ab	34 abcd	252.5 b
KFD-699-01	424	38 cd	69 bc	88 ab	89 a	85 ab	85 ab	--	53 ab	47 cd	230.1 b
KFD-699-01	212	29 cd	64 c	80 b	85 a	73 b	71 b	--	35 bc	42 bcd	246.7 b
Nontreated	0	0 e	0 d	0 c	0 b	0 c	0 c	--	0 c	51 d	328.0 c

[a]Visual control rated on 0 to 100% scale; 0% (no plant response) to 100% (complete necrosis).

[b]Mean response within a column followed by the same letter do not differ according to Fisher's Protected LSD ($P \leq 0.05$).

[c]No data represented as (--) is due to lack of evaluation.

[d]Nonionic surfactant at 0.25% v/v included with all herbicide applications.

[e]Endothall and 2,4-D are reported as g a.e. ha^{-1}.

[f]Fractional green canopy cover (FGCC) at harvest; 0% (no green canopy cover) to 100% (complete green canopy cover).

Table 6-3. Visual percent control, fractional green canopy cover, and dry weights of giant salvinia following foliar herbicide applications via backpack and unoccupied aerial application systems in trial two.

		Weeks After Treatment[a,b,c]									
Herbicide[d]	Rate[e]	1	2	3	4	5	6	7	8	FGCC[f]	Dry Weight
Backpack	g a.i. ha^{-1}	----	----	----	----	%	----	----	----	%	g
Metsulfuron-methyl	21	82 a	84 b	98 a	97 a	91 ab	--	10 c	6 d	53 a	336.8 a
Metsulfuron-methyl	42	88 a	95 a	100 a	100 a	99 a	--	88 a	86 ab	19 c	226.2 a
Flumioxazin	210	90 a	95 a	99 a	98 a	96 ab	--	73 ab	50 c	34 bc	283.8 a
Glyphosate	4539	87 a	97 a	99 a	100 a	99 ab	--	96 a	94 a	17 c	256.8 a
Flumioxazin + Glyphosate	210 + 4539	88 a	90 ab	90 a	65 ab	54 bc	--	5 c	3 d	51 ab	255.3 a
Carfentrazone	67	88 a	89 ab	92 a	53 b	48 c	--	2.5 c	1 d	51 ab	279.8 a
Carfentrazone + Penoxsulam	67 + 70	93 a	94 ab	99 a	97 a	94 ab	--	64 b	58 bc	29 c	314.7 a
Diquat	3136	87 a	90 ab	100 a	99 a	99 ab	--	91 a	87 ab	15 c	214.2 a
Nontreated	0	0 b	0 c	0 b	0 c	0 d	--	0 c	0 d	60 a	334.8 a
Unoccupied Aerial Application System[g]											
Carfentrazone	67	80	74*	71*	55	48	--	0	0	45	252.7

[a]Visual control rated on 0 to 100% scale; 0% (no plant response) to 100% (complete necrosis).

[b]Mean response within a column followed by the same letter do not differ according to Fisher's Protected LSD ($P \leq 0.05$).

[c]No data represented as (--) is due to lack of evaluation.

[d]Non-ionic surfactant at 0.25% v/v included with all herbicide applications.

[e]Glyphosate is reported as g a.e. ha^{-1}.

[f]Fractional green canopy cover (FGCC) at harvest; 0% (no green canopy cover) to 100% (complete green canopy cover).

[g]Asterisk following the metric indicates the mean response by application method differs for carfentrazone using Student's t-test ($\alpha = 0.05$).

Table 6-4. Visual percent control, fractional green canopy cover, and dry weights of giant salvinia following select follow-by foliar herbicide applications overtop plants treated six weeks prior with fluridone. Applications were made via backpack and unoccupied aerial application systems in trial three.

		Weeks After Treatment[a,b,c,d]										
Herbicide[e]	Rate	0	1	2	3	4	5	6	7	8	FGCC[f]	Dry Weight
Backpack	g a.i. ha^{-1}	------------------				%				------------------	%	g
Flumioxazin	210	18 a	92 a	99 a	94 a	91 a	--	38 a	--	0 a	68 a	217.0 a
Carfentrazone	67	15 a	92 a	98 ab	92 a	83 a	--	35 a	--	0 a	70 a	178.0 a
Diquat	3136	16 a	96 a	97 b	90 a	83 a	--	43 a	--	0 a	65 a	144.7 a
Nontreated	0	18 a	0 b	0 c	0 b	0 b	--	0 b	--	0 a	75 a	357.6 a
Unoccupied Aerial Application System[g]												
Carfentrazone	67	15	85	88*	83*	76	--	--	--	0	63	293.5

[a]Visual control rated on 0 to 100% scale; 0% (no plant response) to 100% (complete necrosis).

[b]Ratings at zero weeks after treatment represent pretreatment plant status following an in-water application of fluridone 6 weeks prior to trial initiation.

[c]Mean response within a column followed by the same letter do not differ according to Fisher's Protected LSD ($P \leq 0.05$).

[d]No data represented as (--) is due to lack of evaluation.

[e]Non-ionic surfactant at 0.25% v/v included with all herbicide applications.

[f]Fractional green canopy cover (FGCC) at harvest; 0% (no green canopy cover) to 100% (complete green canopy cover).

[g]Asterisk following the metric indicates the mean response by application method differs for carfentrazone using Student's t-test ($\alpha = 0.05$).

Table 6-5. Visual percent control, fractional green canopy cover, and dry weights of giant salvinia as effected by herbicide and carrier volume following select foliar applications in greenhouse mesocosm settings.

		Weeks After Treatment[a,b]					
Carrier Volume[c]	Rate[d]	1	2	3	4	FGCC[e]	Dry Weight
L ha^{-1}	g a.i. ha^{-1}	----------------------%			----------------------	%	g
Diquat	**3136**						
935		99 a	100 a	100 a	100 a	0 a	0.0 a
140		96 ab	99 ab	100 a	100 a	0 a	0.0 a
23		66 d	88 c	91 bc	94 ab	12 a	0.1 a
Flumioxazin	**210**						
935		85 bc	90 c	94 ab	91 abc	29 b	0.7 b
140		81 c	91 bc	92 abc	87 bc	29 b	0.8 b
23		83 c	83 c	85 cd	73 d	46 cd	1.6 de
Glyphosate	**4539**						
935		56 d	70 d	80 d	82 cd	40 bc	1.1 bc
140		10 e	40 e	62 e	74 d	60 e	1.3 cd
23		8 e	12 f	31 f	48 e	59 de	2.0 e
Nontreated	**0**	0 f	0 g	0 g	0 f	96 f	5.7 f

[a]Visual control rated on 0 to 100% scale; 0% (no plant response) to 100% (complete necrosis).

[b]Mean response within a column followed by the same letter do not differ according to Fisher's Protected LSD ($P \leq 0.05$).

[c]Non-ionic surfactant at 0.25% v/v included with all herbicide applications.

[d]Glyphosate is reported as g a.e. ha^{-1}.

[e]Fractional green canopy cover (FGCC) at harvest; 0% (no green canopy cover) to 100% (complete green canopy cover).

FIGURES

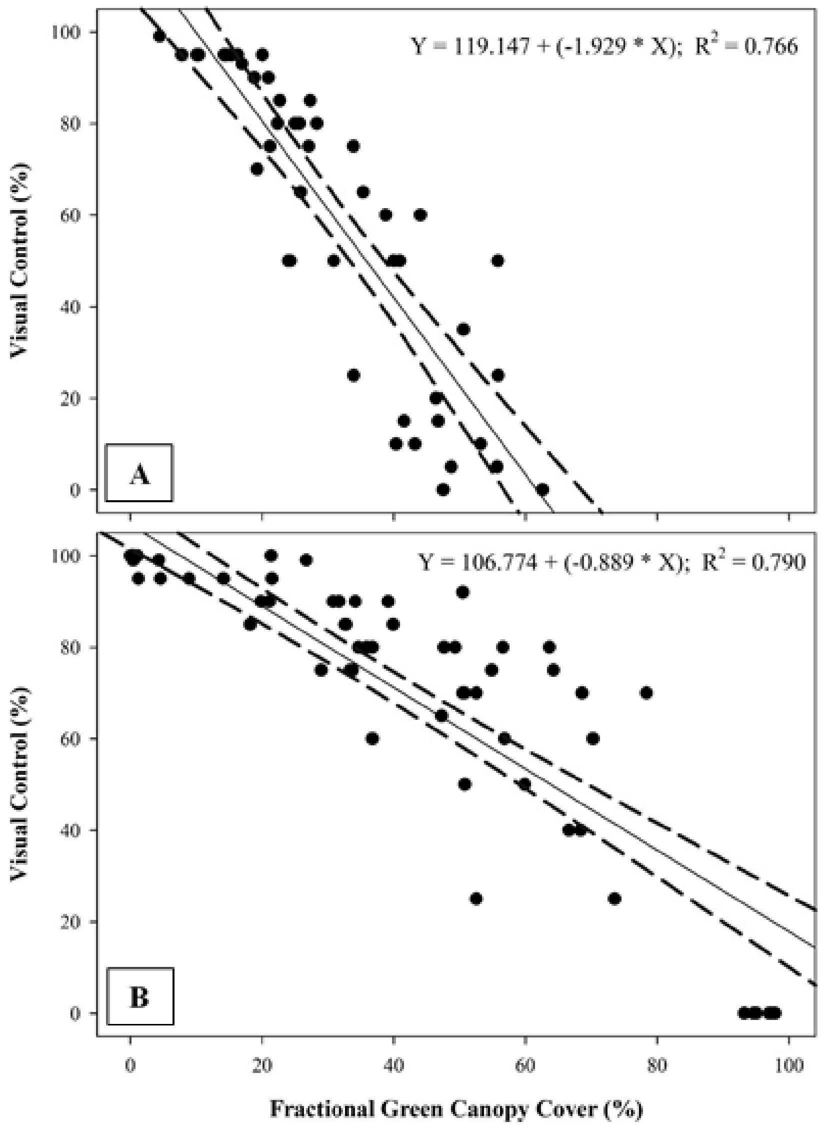

Figure 6-1. Linear regression relationships between calculated fractional green canopy cover (FGCC) and visual percent control estimates of giant salvinia (*Salvinia molesta*) treated with experimental and aquatic herbicides in field trials 1 and 2 (8 weeks after treatment) (A), and the effect of carrier volume and selected herbicides in the greenhouse trial (4 weeks after treatment) (B). Plotted point values represent calculated FGCC and visual percent control of giant salvinia within respective experimental units for each trial. The regression models are depicted as solid black lines with dashed lines representing 95% confidence intervals.

CHAPTER 7: Performance of unoccupied aerial application systems for aquatic weed management: Two novel case studies

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Abstract

Unoccupied aerial application systems (UAAS) are gaining popularity for weed management to increase applicator safety and to deliver herbicide treatments where treatment sites limit ground-based spray equipment. Several studies have documented UAAS application strategies and procedures for weed control in terrestrial settings, yet literature describing remote spray technology for use in aquatics remains limited. Currently, applicators seek guidance for UAAS deployment for aquatic weed management to overcome site access restrictions, environmental limitations, and to improve ground-based applicator safety in hazardous treatment scenarios. In the present case studies, we evaluate a consumer available UAAS to deliver the herbicide, florpyrauxifen-benzyl, as both foliar and directed in-water spray applications. Results from the first case study showed the invasive floating-leaved plant, yellow floatingheart (*Nymphoides peltata*), was visually controlled 80 to 99% by 6 wk after treatment (WAT) following UAAS foliar herbicide treatments. The second case study demonstrated UAAS directed in-water herbicide application from UAAS reduced variable-leaf watermilfoil (*Myriophyllum heterophyllum*) visible plant material by 94% at 5 WAT. Data from both case studies indicate UAAS can provide an effective and efficient treatment strategy for floating-leaved and submersed plant control among common herbicide treatment scenarios. Future

integration of UAAS in aquatic weed control programs is encouraged, especially among smaller treatment sites (≤ 4 ha) or where access limits traditional spray operations.

Introduction

Active management of invasive aquatic plants (AIP) like Eurasian watermilfoil [*Myriophyllum spicatum* L.] and exotic floatinghearts [*Nymphoides* spp.] is required to sustain native species diversity, mitigate ecologically damaging effects from resource competition (Madsen and Sand-Jensen 1991; Zhang and Boyle 2010), and minimize habitat degradation (Houlahan and Findlay 2004; Madsen 2014). However, AIP regularly threaten ecosystem services, impede recreational opportunity, obstruct drainages and irrigation schedules, and hinder hydroelectric power generation (Langeland 1996; Wilcove et al. 1998). While not as common, the management of native aquatic plants (e.g., eelgrass [*Vallisneria americana* Michx.] and spatterdock [*Nuphar lutea* (Aiton) W.T. Aiton] does occur, as some native species can quickly reach nuisance levels (Gettys 2019). Management typically integrates several weed control strategies (Gettys et al. 2020), though herbicides are widely used to selectively target AIP incursions (Nelson et al. 2001; McFarland et al. 2004). In the United States, more than $100 million is allocated for aquatic plant control annually (Rockwell 2003; Pimentel et al. 2005).

Aquatic weed control programs are frequently paired with monitoring surveys to gauge plant presence and breadth prior to management activity. Similarly, plant surveys provide quantitative assessment of weed control efforts (Madsen and Wersal 2017). To date, monitoring remains the most effective method to combat deleterious AIP, especially when deploying early detection and rapid response (EDRR) tactics (Westbrooks 2004). Traditional assessment methods like point-intercept, destructive biomass harvests, or nondestructive hydroacoustic measurements (for submersed plants) are effective for estimating AIP abundance and distribution

(Madsen 1999; Johnson and Newman 2011; Valley et al. 2015; Howell and Richardson 2019). However, these evaluations generally require boating access, skilled labor (e.g., species identification; boating and diving proficiency), and substantial time to meet desired spatial coverage. Remote sensing data from satellite and airborne sensors have supported aquatic plant observations across a variety of species and landscapes to overcome traditional survey limitations (Ackleson and Klemas 1987; Nelson et al. 2006; Santos et al. 2016). Still, the limited spatiotemporal resolution of open-sourced satellites (e.g., Landsat 7 ETM+ offers 30 m^2 pixels; imagery every ~ 15 d) and the associated costs of airborne missions often confines the utility of these platforms within small waterbodies. Recently, managers have deployed consumer available small unoccupied aerial systems (sUAS) to accompany in-field surveys to enhance mapping capability (Kislik et al. 2020). Image data from sUAS generate mapping elements that provide superior spatial resolution (<1 to 5 cm^2 pixels) at user defined temporal resolutions (hrs to days). Similarly, sUAS implementation is cost-effective (Lomax et al. 2005), and generally reduces survey time and labor requirements (Fitzpatric 2015; Nowak et al. 2018). Several studies have revealed the convenience and accuracy of low-altitude (<122 m above ground level; AGL) true-color sUAS usage for emergent, floating, and submersed weed detection (Chabot et al. 2016; Hill et al. 2017; Kislik et al. 2020; Anderson et al. 2021).

In addition to providing a platform for optical imagers, some sUAS offer the opportunity to remotely deliver herbicide applications (Göktoğan et al. 2009; Milling 2018; Hunter et al. 2020; Rodriguez et al. 2022). These sUAS, commonly referred to as unoccupied aerial application systems (UAAS), continue to gain popularity where herbicide treatments are limited with ground-based spray equipment (Xue et al. 2016; Lan et al. 2017; Wang et al. 2019). Benefits of UAAS include improved safety of herbicide application (i.e., personnel have reduced exposure to spray solutions), and the absence of an on-board pilot reduces the inherent risks associated with traditional aerial application techniques (Sheets 2018; Vu et al. 2019). Further, UAAS have the capability to navigate small (e.g., ≤ 4 ha) application sites that would be costly or prohibited with a helicopter or plane (He 2018; Otto et al. 2018), and allow for targeted spray missions intended for site-specific weed management (e.g., spot treating) (Hunter et al. 2020; Pathak et al. 2020).

Positive results have ensued when deploying UAAS for site-specific weed management in terrestrial systems (Ahmad et al. 2020; Hunter et al. 2020; Martin et al. 2020), and there is an opportunity to adopt similar spray technology for use in aquatics. Because aquatic weed managers work in two- and three-dimensional spaces (i.e., foliar and in-water herbicide treatments, respectively), there is a niche for UAAS integration to meet management needs and increase applicator safety. In Australia, an autonomous rotary-wing UAAS used dye as a herbicide surrogate to simulate the spray of alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.] and giant salvinia [*Salvina molesta* D. Mitch] to demonstrate a proof-of-concept model (Göktoğan et al. 2009). However, no studies currently exist describing UAAS strategies and recommended spray application parameters designed for aquatic weed management with herbicides. Nevertheless, applicators express interest in UAAS to meet common environmental complexities (e.g., shallow water; stumps; lack of boat launch facilities) which is indicative of aquatic herbicide applications. There remains a clear need to investigate the performance of herbicides delivered from UAAS to guide future aquatic weed management decisions.

Currently, there are sixteen herbicides available for aquatic weed management in the United States (Schardt and Netherland 2020). While many of the available herbicides provide reliable control of AIP (Enloe et al. 2022), some may offer more suitable characteristics for

UAAS applications than others do. In 2018, the auxin herbicide florpyrauxifen-benzyl was registered as two different formulations (emulsifiable and soluble concentrates) for aquatic site applications. Florpyrauxifen-benzyl is considered a reduced risk herbicide (USEPA 2017), and has a lower use rate (~100 times less) than the precursor registered aquatic auxin herbicides, 2,4-D and triclopyr. In mesocosm and field screenings, florpyrauxifen-benzyl has displayed high levels of efficacy on submersed, emergent, and floating-leaved AIP, including *M. spicatum*, hydrilla [*Hydrilla verticillata* (L.f.) Royle], parrotfeather watermilfoil [*Myriophyllum aquaticum* (Vell.) Verdc.], and crested floatingheart [*Nymphoides cristata* (Roxb.) O. Ktze.] (Netherland and Richardson 2016; Richardson et al. 2016; Beets and Netherland 2018; Sperry et al. 2021; Howell et al. 2022). Since commercially available UAAS have tank volumes typically ≤ 10 L to meet Federal Aviation Administration regulations (i.e., FAA 14 CFR Part 107), the low use rates of florpyrauxifen-benzyl make it an excellent candidate herbicide for preliminary UAAS evaluations.

In the present studies, we evaluate the performance of UAAS to remotely deliver florpyrauxifen-benzyl herbicide to floating-leaved and submersed vegetation for targeted weed control. We hypothesize herbicide applications made with UAAS will demonstrate similar efficacy expected of ground-based applications and offer an additional spray strategy to manage aquatic weeds in sites having limited access. The objectives were to: 1) determine the feasibility of UAAS in common aquatic weed management scenarios, 2) evaluate if UAAS provide an effective application for target weed control, and 3) identify application constraints that exist with UAAS herbicide delivery technology in aquatics.

Materials and Methods

Case Study 1: Floating-leaved weed control using foliar UAAS techniques.

In the summer of 2020, collaboration efforts with the North Carolina Department of Agriculture aimed to eradicate yellow floatingheart [*Nymphoides peltata* (S.G. Gmel.) Kuntze] from a residential dewatered pond in Lee Co., North Carolina (35.4317°N, 79.1987°W) using remotely delivered foliar herbicide applications. Following a series of tropical storms from 2016 to 2018 that led to dam failure, the watered surface of the pond was reduced 80 to 90% of the original area. The only remaining water averaged 0.48 m (± 0.31 SD) in depth during initial site inspections. Due to woody (e.g., sweetgum [*Liquidambar styraciflua* L]; willow [*Salix* spp.]) and herbaceous (e.g., cattail [*Typha latifolia* L]; bulrush [*Scirpus cyperinus* (L.) Kunth]) vegetation encroachment and limited site access, traditional ground-based herbicide application options were not feasible. Therefore, herbicide applications were designated using a remotely piloted UAAS to selectively treat the *N. peltata* infestation.

Prior to herbicide application, imagery was collected with a DJI Phantom 4 Advanced (DJI, Shenzhen, China) sUAS at 60 m above ground level (AGL) using the flight planning application, Pix4D Capture (Pix4D SA, Prilly, Switzerland), to locate and map *N. peltata* infestations within the pond. Individual images were stitched together with Agisoft Photoscan Metashape 1.5.1 (Agisoft LLC, St. Petersburg, Russia) to generate an orthomosaic GeoTIFF having a ground sampling distance (GSD) of 1.6 cm pixel^{-1}. The georeferenced pretreatment orthomosaic was then imported into QGIS 3.14.16 (QGIS 2022) under a projected coordinate system (EPSG: 32617) to visually reference areas within the pond requiring herbicide treatment. Digital polygons were generated around *N. peltata* populations that allowed aerial access for UAAS treatment (i.e., areas devoid of heavy tree cover). Georeferenced points from ground-based surveys were used to validate plant presence and cover within the aerial imagery. In total, four discrete *N. peltata* infestations were identified within the pond that totaled 0.125 ha (Figure 1; Table 1).

Herbicide applications were initiated 15 July 2020 using a DJI Agras MG-1 octocopter UAAS (DJI, Shenzhen, China). Florpyrauxifen-benzyl (ProcellaCOR SC, SePRO Corp., Carmel, IN, USA), was applied at 58.3 g a.i. ha^{-1} (i.e., 2 Prescription Dose Units; PDU) and methylated seed oil (MSO) was included at 1.0 % v/v. Carrier volume was based on the minimum requirements of the herbicide label at the time of application (Anonymous 2020) and nozzle selection was determined from prior research evaluating spray deposition from the identical UAAS model deployed in the present study (Hunter et al. 2020). Specific flight and treatment parameters are provided in Table 1. Where treatment zones permitted, applications were conducted using the UAAS integrated autonomous spray function. Autonomous operations were arranged as 3.0 m spaced tracks following a serpentine spray pattern which worked off an A-B line set by the remote pilot. All other spray operations were manually piloted using comparable flight parameters to the autonomous spraying to achieve the desired application rate. All herbicide applications occurred when wind speeds were 0.9 to 3.5 ms^{-1}. There was a 24 hr rain free period following the aerial herbicide application.

Evaluations of plant vigor were made at 0, 2, and 6 wk after treatment (WAT) using ground-based surveys coupled with aerial imaging missions from the sUAS using the same flight plans conducted for pretreatment measures. Aerial images from each evaluation time point were stitched to create a georeferenced orthomosaic using methods previously described. Imagery from the subsequent evaluation periods (i.e., post-treatment) were georectified to the pretreatment image using the QGIS Georefrencer toolbox and ground control points (N = 4) to provide image alignment for spatiotemporal analyses. Orthomosaic images representing each evaluation were evaluated using 1.0 m^2 georeferenced digital quadrats (N = 4 per treatment zone; N = 16) to provide spatiotemporal visual density estimates of 0% (no plant cover) to 100% density (complete plant cover). When applicable, injury symptoms to non-target species was recorded using qualitative methods. Collected data were subject to ANOVA under the *agricolae* package in RStudio (R Core Team 2020). When significant effects among evaluation periods were detected, means were separated using Tukey's HSD test ($\alpha = 0.05$).

Case Study 2: Submersed weed control using directed in-water UAAS techniques.

Field experiments were initiated summer 2020 to evaluate the performance of directed in-water herbicide application techniques from UAAS for variable-leaf watermilfoil [*Myriophyllum heterophyllum* Michx.] control within a farm pond in Moore Co., North Carolina (35.3184°N, 79.4010°W). *Myriophyllum heterophyllum* had become well established within the pond, which severely limited recreation and irrigation activity by the landowner. Heavy tree cover around- and dense vegetation within the pond restricted shoreline and watercraft herbicide application, respectively. Prior herbicide treatments to control *M. heterophyllum* in the pond were reported to be ineffective because of poor accessibility, resulting in inadequate spray coverage from the shoreline. This study was developed to remotely deliver herbicide to a nuisance *M. heterophyllum* population to evaluate if adequate plant control could be achieved using UAAS techniques.

Using methods described in previous studies (Madsen 1999; Valley et al. 2015; Madsen and Wersal 2017), a point-intercept kayak survey ensued prior to herbicide treatment to gauge initial *M. heterophyllum* dynamics and determine treatment parameters needed to develop herbicide dilution rates. The survey additionally used a Lowrance Hook (Navico Inc., Tulsa, OK, USA) echosounder and a 200-kHz transducer with a 20° beam angle (10 pings s^{-1}) to passively record depth and submersed plant signatures. Acoustic measurements spanned the length of the pond in a serpentine pattern with tracks spaced approx. 5 m apart. Acoustic data was recorded to the echosounder using a 64-gigabit secure digital card. Logged data files (.sl2) from the completed survey were uploaded to BioBase 5.2 (Navico Inc., Egersund, Norway) cloud-processer to calculate the bathymetry of the pond, the spatial extent of submersed plants, and estimate plant biovolume (i.e., percentage of the water column occupied by plants).

Aerial imagery was collected at 46 m AGL, using analogous methods as described for case study one, to locate and map surface level *M. heterophyllum* within the pond prior to treatment. Recorded images were stitched using procedures previously described to create an orthomosaic image having a GSD of 1.37 cm $pixel^{-1}$. Pretreatment imagery was imported into QGIS under a projected a coordinate system (EPSG: 32617) to identify the locations within the pond requiring herbicide treatment. Georeferenced point locations and biovolume estimates from the kayak survey were used to validate plant incidence observed from the aerial imagery. A digital polygon was created around a section of the primary *M. heterophyllum* population to serve as the designated treatment zone. The estimated surface coverage of the treatment zone was 0.13 ha (Figure 2; Table 1). An area approx. 50 m adjacent the treatment zone, which also contained *M. heterophyllum*, served as a nontreated reference.

Directed in-water herbicide applications were initiated 15 July 2020 using a DJI Agras MG-1 octocopter UAAS modified to dispense the spray solution through a single fertilizer nozzle (Table 1). This nozzle was selected for the reduced potential of spray drift compared to conventional nozzles commonly found on UAAS (Hunter et al. 2020), and the deposition of the solid stream was hypothesized to increase spray penetration through surface weed canopies.

Florpyrauxifen-benzyl was aerially applied at a target concentration of 9.6 µg a.i. L^{-1} (i.e., 1 PDU). The UAAS dispensed the herbicide solution to the water-column using a manual operation flight mode in a serpentine pattern within the selected treatment zone. Wind speed averaged 9.6 km hr^{-1} at the time of aerial herbicide application.

Plant assessments were made at treatment, and at 1, 2, and 5 WAT using kayak surveys coupled with aerial imaging missions from the sUAS using identical flight parameters conducted during pretreatment evaluations. Additionally, low-altitude (3 m AGL) sUAS point photographs were captured at each evaluation period to provide discrete geolocations for visually assessing plant symptomology to the herbicide treatment (e.g., epinasty; chlorosis and necrosis). Flight plan images from each evaluation time point were stitched to create a georeferenced orthomosaic using methods formerly defined. Orthomosaic images from each evaluation were then georectified to the pretreatment imagery using methods previously described, where ground control points (N = 4) were used to align the image rasters for spatiotemporal analyses. Plant response to the herbicide treatment was evaluated using 3.0 m^2 georeferenced digital quadrats (N = 4) to provide spatiotemporal visual density estimates of 0% (no plants visible) to 100% density (complete plant surface coverage). When applicable, injury symptoms to non-target species was recorded using qualitative methods. Collected data were subject to ANOVA in RStudio to determine the main effect of the UAAS herbicide application on treated plant density. Where significant effects occurred, evaluation periods were separated from the nontreated control plot using Student's t-test ($\alpha = 0.05$).

Results and Discussion

Due to the inherent environmental complexities within the application sites for both case studies, only visual control data from sUAS imagery missions is reported for target plant response to the UAAS herbicide treatments. Data from both case studies is presented independently.

Case Study 1: Floating-leaved weed control using foliar UAAS techniques.

At treatment initiation, treatment zone quadrats had >92% *N. peltata* visual coverage (Figure 3). Any *N. peltata* plant populations in community with emergent species (e.g., *T. latifolia*) outside of the targeted treatment zones is not reported on due to the inability for aerial image detection (i.e., not visible from the sUAS imagery due to surrounding erect canopy coverage). The most northern treatment zone (Figure 1), also contained the floating-leaved species white water-lily [*Nymphaea odorata* (Aiton)], which is commonly considered a native pest plant.

Foliar herbicide applications made from UAAS significantly reduced ($P < 0.0001$) *N. peltata* density by ≥ 99% at 2 WAT (Figure 3). Rapid plant response to florpyrauxifen-benzyl was expected for *N. peltata*, as initial plant screenings showed the akin species, *N. cristata*, to be highly sensitive to the herbicide at concentrations ranging 1 to 3 $\mu g\ L^{-1}$ within days after treatment (DAT) (Netherland and Richardson 2016). The non-target species, *N. odorata* was also readily controlled and remaining leaves displayed chlorosis and necrosis at 2 WAT, with complete control observed at the 6 WAT evaluation (data not shown). At 6 WAT, varying levels of *N. peltata* regrowth was detected among three of the four treatment zones. Daughter plants (i.e., immature *N. peltata*) were detectable from aerial imagery at 6 WAT and covered <1 to 20% of the water's surface within sampling quadrats (Figure 3). Since *N. peltata* reproduces both vegetatively and sexually (Markovich et al. 2020), it is likely that observed *N. peltata* plants within treatment zones experiencing regrowth occurred from either stolon fragments or prior seed production rather than lack of control following herbicide application 6 wk prior. A follow-by treatment would have benefitted eradication efforts in the current study and future UAAS operations seeking *N. peltata* control should consider sequential applications as the herbicide label permits (Anonymous 2022).

In a 28 d outdoor mesocosm study, *N. cristata* aboveground biomass was completely controlled following static exposure of florpyrauxifen-benzyl at 24 µg L^{-1} (Beets and Netherland 2018) and while belowground biomass was reduced, it was not significant ($\alpha = 0.05$) from that of the nontreated control plants. Nevertheless, Haug et al. (2021) observed high belowground concentrations of florpyrauxifen-benzyl for *N. cristata* in a ^{14}C study evaluating the absorption and translocation of the herbicide. *Nymphoides peltata* foliar treatment response data from the present case study aligns well with these former findings of florpyrauxifen-benzyl applications made in-water to *N. cristata*. However, it is likely that overspray from UAAS foliar applications that would provide in-water herbicide activity was limited due to the shallow water conditions within *N. peltata* treatment zones. Ultimately, environmental conditions likely reduced any residual in-water activity of florpyrauxifen-benzyl since the herbicide rapidly degrades through photolysis (<1 d) and strongly binds to sediment (USEPA 2017; Heilman and Getsinger 2018). Future research investigating *N. peltata* control from foliar application techniques would be beneficial to evaluate the fate of the herbicide under varying depth gradients to reduce belowground storages (i.e., stolons) capable of successional growth.

Several treatment considerations transpired which will benefit future management action for floating-leaved management. Because of tall surrounding vegetation (e.g., *T. latifolia*) within the infestation site, traditional backpack application techniques would have likely limited foliar application to *N. peltata* targets. Similarly, backpack applications from ground crew would have required more than one applicator to achieve effective and efficient herbicide treatments. While access to the edge of the *N. peltata* pond was largely restricted by tree cover, UAAS takeoff and landing was not constrained by the complex environmental conditions, as an improvised landing zone was easily constructed by compressing bulrush (*Scirpus* spp.) in a circular configuration (1.5x width of UAAS wingspan). The ability to remotely treat *N. peltata* under these environmental constraints further highlights the utility as another aquatic plant management application method. Further, the efficiency of UAAS demonstrated that a remote herbicide application could occur within expected timeframes of ground applications while also safely removing crew from environmental hazards (e.g., snakes) and contact with herbicide during application (Table 1). Notably, application efficiency could be improved upon as UAAS technologies advance, especially when integrating RTK UAAS platforms that allow for more precise autonomous spray missions (Zhang et al. 2020). In this case, herbicide applications would have minimal input from the remote pilot since environmental parameters would be pre-mapped and uploaded to the UAAS platform, which would increase the sprayer's overall accuracy and efficiency potential (Hunter et al. 2020).

Case Study 2: Submersed weed control using directed in-water UAAS techniques.

At the time of the UAAS directed in-water treatment, *M. heterophyllum* was topped-out (i.e., 100% coverage of the surface of the water column) within the selected treatment zone. The native floating-leaved species, watershield [*Brasenia schreberi* (J.F. Gmel.)], covered approx. 50% of the treatment zone at the time of herbicide application. Though not specifically targeted for, *B. schreberi* injury was documented when appropriate since the plant can be a nuisance within small ponds due to dense growth formations (Weldon et al. 1969). Plants within the nontreated control plot retained vigor with no signs of injury throughout the 5 wk study duration.

At 1 WAT, directed in-water florpyrauxifen-benzyl applications made from UAAS did not significantly ($P = 0.391$) reduce visual surface level coverage of *M. heterophyllum* (Figure 4). However, canopy collapse was initiated by 1 WAT, and terminal epinasty of plant shoots and inflorescences was apparent (data not shown). In greenhouse settings, *M. heterophyllum* treated with florpyrauxifen-benzyl at 0.3 to 81 µg L^{-1} showed rapid stem growth and epinasty within 1 WAT (Richardson et al. 2016), which aligns well with plant response in the present case study. Additionally, *B. schreberi* within the treatment zone was chlorotic and displayed leaf curling at 1 WAT (data not shown). These observed injury symptoms at 1 WAT are common among plants sensitive to auxin herbicides like florpyrauxifen-benzyl (Grossmann 2010; Howell et al. 2021). By 2 WAT, a significant treatment effect was detected ($P = 0.036$), where visual *M. heterophyllum* density was reduced by 45% compared to the pretreatment measures (Figure 4). Low-altitude image capture and kayak surveys visually indicated *M. heterophyllum* had also begun fragmenting by 2 WAT (data not shown). *Brasenia schreberi* response at 2 WAT showed an overall decline in plant vigor (e.g., chlorotic and necrotic leaves), and by 5 WAT the plant's coverage was reduced approx. 80% that of the initial survey (data not shown). These observations suggest that in-water exposures of florpyrauxifen-benzyl at 9 µg L^{-1} readily controls *B. schreberi*. At the final evaluation 5 WAT, *M. heterophyllum* imagery showed a significant decline in density ($P < 0.0001$), as only 6% of the initial plant material remained detectable within the sampling quadrats (Figure 4). A former study evaluating *M. heterophyllum* control with florpyrauxifen-benzyl at concentrations ≥ 9 µg L^{-1} observed ≥ 90% plant biomass inhibition at 4 WAT (Richardson et al. 2016), which corroborates the high level of plant suppression experienced in the present study.

While additional evaluations did not occur beyond 5 WAT, *M. heterophyllum* response to the directed in-water treatments of florpyrauxifen-benzyl from UAAS were considered efficacious for targeted submersed plant control. In a 4 wk mesocosm study, the lowest observed effect concentration for *M. heterophyllum* was 9 µg L^{-1} of florpyrauxifen-benzyl (Richardson et al. 2016), which is analogous to the target herbicide concentration utilized in the present case study (9.6 µg a.i. L^{-1}). Additional studies investigating florpyrauxifen-benzyl efficacy on invasive Myriophyllum spp. indicate low herbicide concentrations ($\leq$ 12 µg L^{-1}) at static or short exposure periods (e.g., 3 hr) reduces hybrid watermilfoil [*Myriophyllum spicatum* L. X *Myriophyllum sibiricum* Kom.] biomass ≥ 98% at 30 to 60 DAT (Beets et al. 2019; Mudge et al. 2021). Data from the present study and past studies, suggests high levels of Myriophyllum spp. control is achievable with florpyrauxifen-benzyl deployed under directed in-water UAAS techniques. Additionally, evidence suggests similar aerial herbicide application methods could be implemented to manage native floating-leaved plant pests like *B. schreberi*.

Treatment parameters from the current case study demonstrated directed in-water aerial applications from UAAS provide an efficient and effective option to treat submersed plant targets when water access is restricted (Table 1). The total application time, which included UAAS setup, filling the spray tank, conducting treatment, and UAAS disassembly, occurred faster than would be expected for ground-based spray operations in similar scenarios. Additionally, the ease of application was notable and the ability to treat submersed plants without the need to deploy watercraft improved operational efficiency. In general, application efficiency is highly dependent on the need to refill the spray tank with herbicide solution and exchange batteries for continued flight (UAAS flight duration is approx. 8 to 10 min per battery). Though current battery technology confines potential flight time (Ying et al. 2016) and federal aviation regulations dictate UAAS total weight capacity (i.e., FAA 14 CFR part 107 requires < 24.9 kg at take-off), permitted modifications made to herbicide labels could improve the efficiency of remote spray operations. For example, the current herbicide label for florpyrauxifen-benzyl requires a minimum carrier volume of 46.8 L ha^{-1} for aerial applications (Anonymous 2022). In treatment zones > 1.0 ha, application efficiency would likely increase if the minimum carrier volume requirements were reduced by half (e.g., 23.4 L ha^{-1} volumes requires three less tank fills for a 10 L capacity UAAS). Furthermore, directed in-water application techniques would be expected to achieve target herbicide concentrations regardless of carrier volume. In a submersed field application study evaluating florpyrauxifen-benzyl to suppress dense dioecious *H. verticillata*, the herbicide was shown to rapidly dissipate within 6-hr following treatment (Sperry et al. 2021). Research evaluating ultralow spray volumes with florpyrauxifen-benzyl to submersed plant targets would benefit future direction of directed in-water application strategies with UAAS.

Conclusion

This is the first published field data in the United States describing herbicide efficacy deployed from UAAS for targeted weed control in aquatics. Results from both case studies indicate integrating UAAS for floating-leaved and submersed plant control can provide an efficient and effective alternative to traditional ground-based application strategies. Nevertheless, the efficacy and efficiency of foliar applications made at low-release altitudes (<3 m AGL) will remain highly dependent on target plant distribution, UAAS spray deposition, and surrounding obstructions. The deployment of sUAS to monitor herbicide applications greatly improved the ability to estimate target plant control following herbicide application. Further, the use of sUAS imagery aided in the detection of *N. peltata* populations formerly unidentified using ground-based assessment methods in the first case study and directly supports EDRR strategies. Although the potential exists to incorporate UAAS for aquatic plant management strategies, we recognize that current UAAS regulations and aquatic herbicide labels may restrict the broader integration of remote spray systems for applicators at present. Research investigating the effects of herbicide efficacy at ultralow carrier volumes would improve the utility of UAAS and provide further direction for UAAS in aquatics. Similarly, examining additional directed in-water application strategies (e.g., releasing herbicide in a grid pattern) could increase the overall efficiency of UAAS in larger submersed plant treatment scenarios.

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TABLES

Table 7-1. Unoccupied aerial application system treatment parameters for case studies one and two.

Parameter[†]	**Case Study 1**	**Case Study 2**
Plant target	*Nymphoides peltata*	*Myriophyllum heterophyllum*
Treatment type	Foliar	Directed in-water
Nozzle type	AIXR 11002-VP	FERT SJ3-02-VP
Nozzle number	4	1
Spray angle (°)	0 (vertically down)	0 (vertically down)
Swath width (m)	3	0.9
Release height (m)	3	3
Flow rate (single nozzle; mL min^{-1})	775	1100
Application method	Manual/Autonomous	Manual
Application speed (m s^{-1})	1	2.24
Carrier volume (L ha^{-1})	149.7	46.8
Application area (ha)	0.125	0.13
Treatment time (min)	13	6
Total application time (min)	150	20
Application efficiency (ha hr^{-1})[‡]	~ 0.6	1.3

[†]For both case studies, a DJI Agras MG-1 UAAS was deployed for treatment.

[‡]Application efficiency defined as the potential treatment area achievable in one hour without battery exchanges or refilling the UAAS spray tank.

FIGURES

Figure 7-1. Aerial image map of the *Nymphoides peltata* pond and selected treatment zones for unoccupied aerial application system foliar florpyrauxifen-benzyl applications. Treatment zones are shown in black while the digital sampling quadrats used for pre- and post-treatment visual evaluations are displayed as red polygons (N = 16). The inset image in the legend displays the typical density of *N. peltata* within a mixed community of *Typha latifolia*.

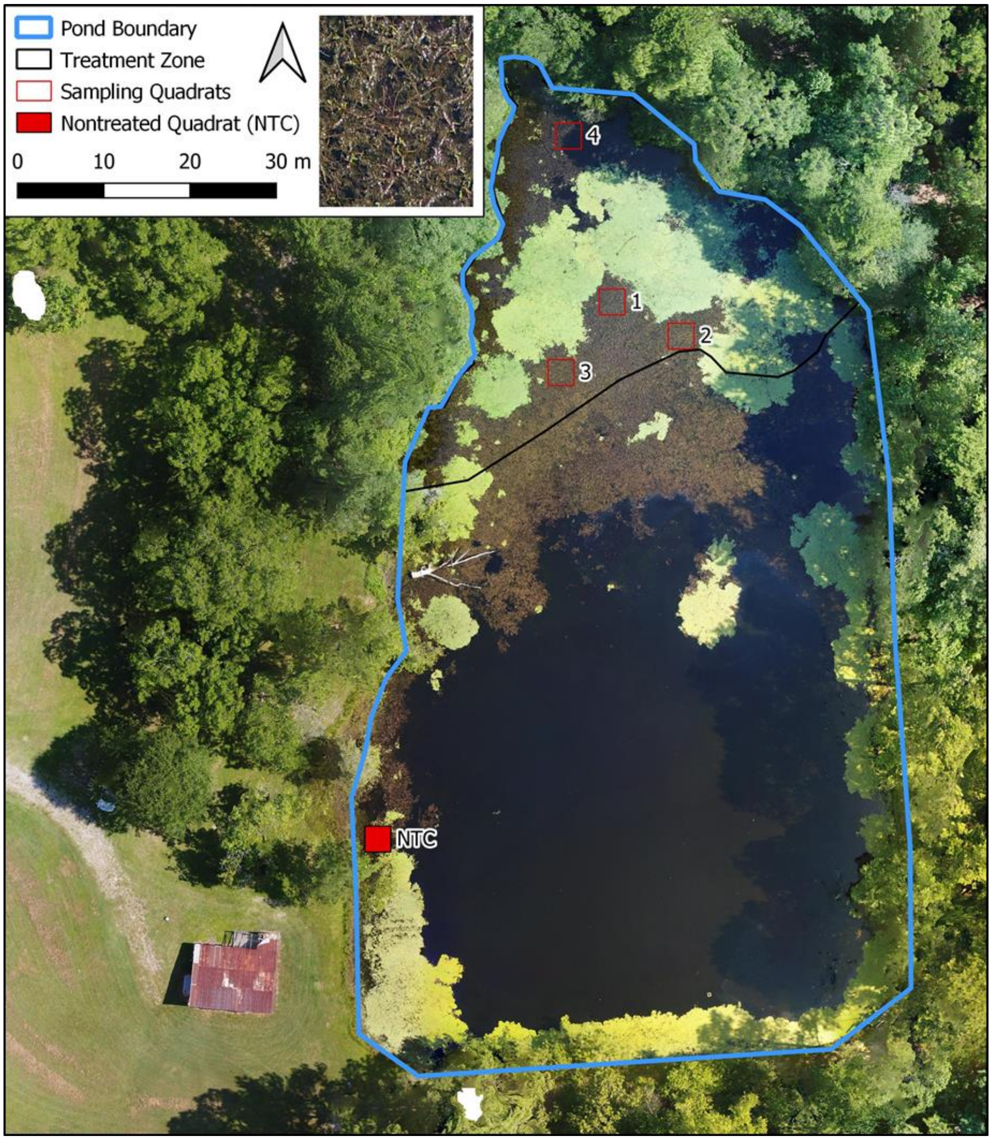

Figure 7-2. Aerial image map of the *Myriophyllum heterophyllum* pond and the selected treatment zone for unoccupied aerial application system directed in-water florpyrauxifen-benzyl application. The treatment zone is shown in black while the digital sampling quadrats used for pre- and post-treatment visual evaluations are displayed as red polygons (N = 4). The nontreated control quadrat (NTC) is displayed as the solid red polygon. Within the pond boundary, dark brown submersed plant growth is *M. heterophyllum* while the bright green floating-leaved patches are *Brasenia schreberi*. The inset image in the legend displays the typical surface level density of *M. heterophyllum* at pretreatment.

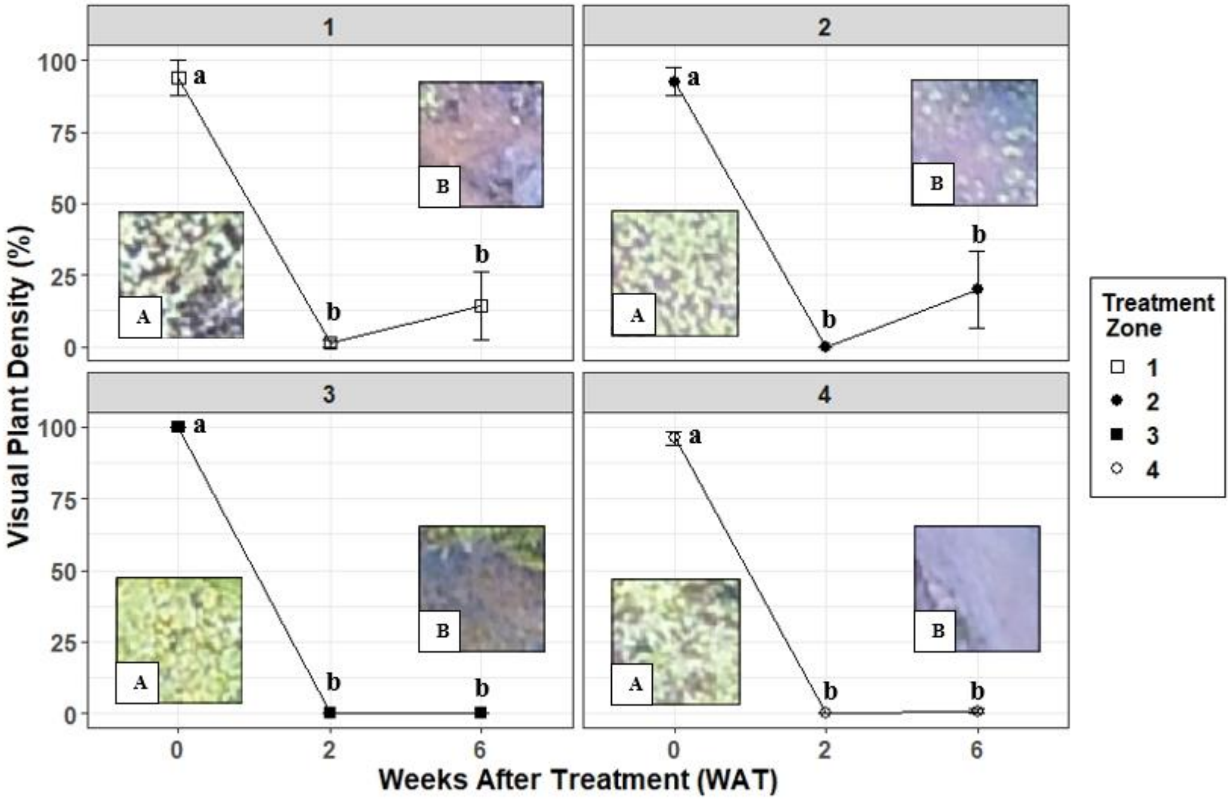

Figure 7-3. The effect of a single florpyrauxifen-benzyl foliar treatment made with an unoccupied aerial application system for *Nymphoides peltata* eradication efforts within select treatment zones. Data describe the visual plant density response to the herbicide treatment within the digital sampling quadrats (n = 4) placed within each treatment zone (mean visual density ± SE; N = 16). Means between evaluation periods with identical letters around the standard error bars are not different according to Tukey's HSD test (α = 0.05). The inset plot images provide a visual example of pretreatment plants (A) and plants treated with herbicide (B) at 6 wk after treatment.

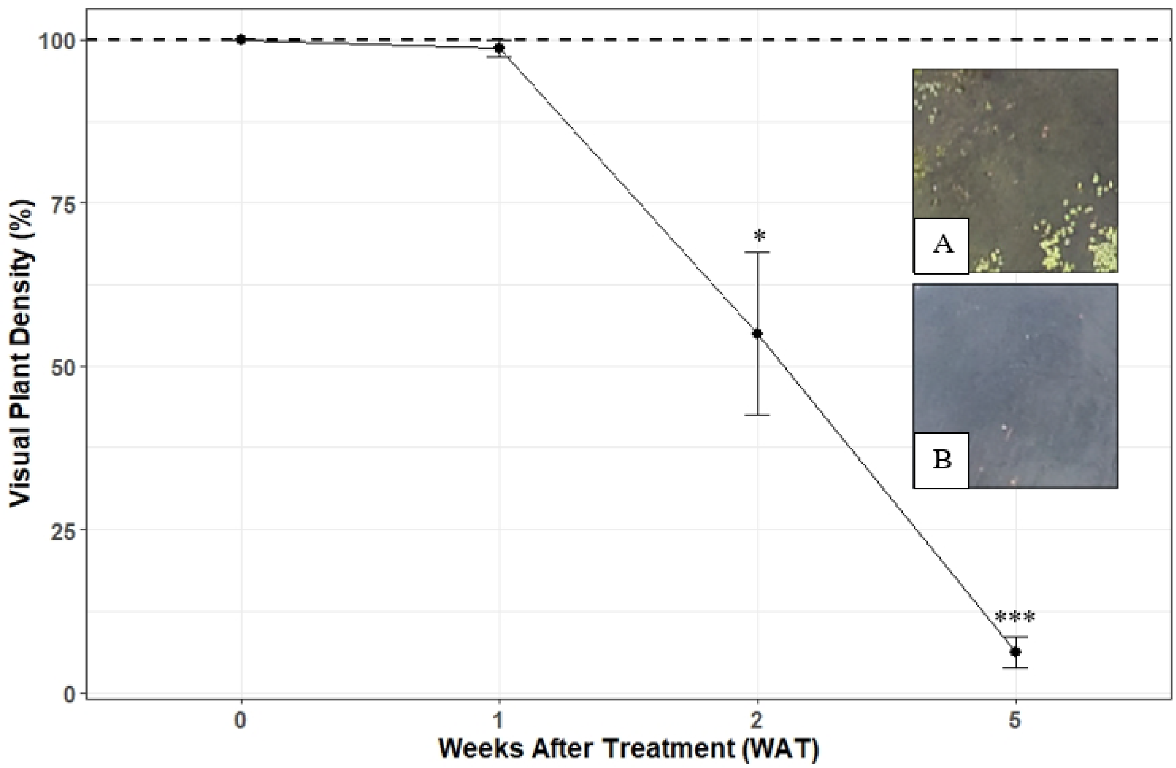

Figure 7-4. The effect of a single directed in-water florpyrauxifen-benzyl application made with an unoccupied aerial application system for *Myriophyllum heterophyllum* control under static conditions. Data describe the visual plant density response to the herbicide treatment within the digital sampling quadrats (mean visual density ± SE; N = 4). The dashed trend line represents the nontreated control plot, while the solid line represents the trend in plant response to herbicide treatment. An asterisk above the standard error bars indicates the detection of a significant interaction between the treated and nontreated control plots according to Student's t-test at $\alpha = 0.05$ ($P < 0.05$ '*'; $P < 0.0001$ '***'). The inset plot images provide a visual example of nontreated plants (A) and plants treated with herbicide (B) at 5 wk after treatment.