

Enhanced Precision of Aerial Herbicide Application for Invasive Species Management

by

Grace Lew-Kowal

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Author's declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Statement of contributions

This thesis work is my intellectual property and I take sole responsibility for it. I am the sole author of chapters 1 and 5, which are not intended for publication. I intend to publish my three data chapters (Chapters 2 – 4), as stand-alone manuscripts. The work for these data chapters was co-conceptualized with Dr. Janice Gilbert and Dr. Rebecca Rooney, and the methodology further refined by input from Dr. Adam Yates and Dr. Derek Robinson. I completed the field work, data curation, formal analysis, visualization, and writing of the original drafts. I acknowledge that Dr. Rebecca Rooney also assisted with project administration, data analysis, and editing the write-up of this thesis document and will have her as a co-author for each of the manuscripts. To recognize these contributions, I use “we” throughout the thesis document.

Abstract

Invasive common reed (*Phragmites australis* ssp. *australis*) has established and dominated in Ontario wetlands for decades. The detrimental effects of *P. australis* invasions on wetland habitats have demanded intervention through aggressive suppression efforts. However, constraints in available control methods to suppress *P. australis* have led to persistence. To improve *P. australis* management in wetlands, we investigated remotely piloted aircraft systems (RPASs) as a precision tool for herbicide application. We applied Habitat® Aqua with a spray-equipped RPAS at selected pilot sites, marking the first-ever application of its kind in Canada. We evaluated the suppression efficacy of RPAS-based herbicide application to *P. australis* and examined the plant community changes one-year after the initial herbicide application. We found a > 99% reduction in live *P. australis* stems, along with reductions in species richness (33%), Shannon-Weiner diversity (73%), Simpson's reciprocal diversity (50%), and Pielou's evenness (73%) in the year following herbicide application. We performed an in-field application experiment to quantify the herbicide deposition of an RPAS-based application through the vertical profile of a *P. australis* canopy, comparing medium and coarse droplet sizes. We determined that both droplet sizes achieved similar coverage and vertical penetration of herbicide within the *P. australis* canopy. Lateral droplet drift occurred at least 8 m away from the application area with medium droplets diminishing at a faster rate than the coarse droplets. We used remotely-sensed images taken before and after the RPAS-based herbicide application to determine the application accuracy and post-treatment effects. We determined that RPAS-based herbicide application to *P. australis* was on-target 91% of the time. The herbicide drift footprint extended up to 20 m away from the application area, representing a distance that is 96% lower than the label-recommended buffer distance for Habitat® Aqua application with a helicopter.

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1.0 General introduction

1.1 Overview

Wetland managers have struggled for decades to control biological invasions by European *Phragmites australis* ssp. *australis* (Rohal et al., 2018). Despite considerable control efforts, many small, inaccessible *P. australis* patches remain due to constraints in available treatment options. In this thesis, we use a combination of field-based studies and remotely-sensed images to assess the effectiveness of *P. australis* control when herbicides are applied by remotely-piloted aircraft systems (RPAS). We examine the suppression efficacy and application accuracy of RPAS-based herbicide application to *P. australis* in various Ontario wetlands, which represent environments where traditional *P. australis* control methods have been limited.

1.2 *Phragmites australis* ssp. *australis* invasions in wetlands

Wetlands make up nearly 35 million hectares of Ontario (Ministry of Natural Resources and Forestry, 2021) and provide diverse ecosystem services, including habitat for sensitive species, improved water quality, and carbon sequestration (Sierszen et al., 2012). However, the introduction of invasive species can alter these ecosystem functions and negatively impact biodiversity in wetlands (Simberloff et al., 2013). For example, European common reed, *Phragmites australis* ssp. *australis* (hereafter referred to as *P. australis*), is a perennial grass that has established itself in wetlands of the Great Lakes region for decades (Wilcox et al., 2003; Tulbure et al., 2007). Historically, American *P. australis* was a minor component of wetland vegetation in North America, but over the past century a European subspecies has invaded, outcompeting native subspecies and altering habitat structure (Saltonstall, 2002). *Phragmites*

australis is believed to have been introduced to North America through ballast from European ships (Saltonstall, 2002). Early records indicate that *P. australis* spread rapidly along the Atlantic Coast in the mid-20th century, driven by changes in land use and hydrology (Chambers et al., 1999). Since its introduction, *P. australis* has spread rapidly across the continent, facilitated by human activity such as land development and changes in wetland management practices (Catling & Mitrow, 2011). The spread of *P. australis* has been particularly evident in eastern Canada, where it has become a dominant component of wetland ecosystems. For example, satellite imagery from the 2010s found that 11% of emergent wetlands within 10 km of the Great Lakes were dominated by *P. australis* (Bourgeau-Chavez et al., 2015), highlighting its capacity to spread rapidly if left unmanaged. Despite its invasive success, the distribution of *P. australis* is influenced by environmental factors, with nitrogen availability possibly playing a crucial role. While *P. australis* flourishes in high-nitrogen environments, regions with limited nitrogen availability may restrict its growth (Chambers et al., 1999). Future projections suggest that climate change could enable *P. australis* to expand its range northward in Canada (Catling & Mitrow, 2011), though these expansions may be constrained in areas where nitrogen levels are insufficient to support its rapid and dense growth.

Reproducing both vegetatively and sexually, *P. australis* disperses through rhizomes, stolons, and seeds, allowing it to rapidly colonize and form dense monoculture stands (Albert et al., 2015). This growth strategy enables *P. australis* to dynamically expand its range, while its tall (up to 5 m) growth and high carbon assimilation capacity allow it to outcompete native vegetation (Robichaud & Rooney, 2022). These combined factors lead to significant biodiversity loss in wetlands as *P. australis* overtakes areas that native plant communities would historically occupy and competes for resources necessary for native vegetation to survive (Robichaud &

Rooney, 2022). Its ability to establish and spread in new areas is facilitated by a high tolerance to a wide range of environmental conditions, including fluctuations in water levels (Whyte et al., 2008). *Phragmites australis* thrives in the biodiverse wetlands of the Great Lakes, where fluctuating water levels can create a dynamic shift in native species composition (Tulbure & Johnston, 2010). For example, in high-water level conditions, the abundance of emergent and meadow marsh species (e.g., *Pontederia cordata*, *Alisma subcordatum*) decreases, to be replaced by floating and submersed aquatic vegetation (e.g., *Nelumbo lutea*, *Ceratophyllum demersum*), with the opposite being true of low-water conditions (Mortsch et al., 2008).

While more sensitive native species are heavily influenced by varying water depths, *P. australis* is tolerant of these conditions and maintains dominance in wetlands where water level fluctuations often occur. For example, at Long Point Provincial Park low-water conditions in the early 1990's led to a rapid expansion of *P. australis* in areas occupied by meadow marsh communities and was able to persist even as water-levels increased in following years (Wilcox et al., 2003). The replacement of diverse meadow marsh communities to dense, rigid, monoculture stands formed by *P. australis* also reduces habitat quality for many wildlife species, including marsh-nesting birds (Robichaud & Rooney, 2017) and reptiles (Markle & Chow-Fraser, 2018), as their nesting habitats are disturbed or lost. These habitat changes contribute to a decline in species diversity and abundance, further highlighting the ecological threat posed by *P. australis* invasions. Overall, the spread of *P. australis*, coupled with its high environmental tolerance leads to significant ecological disruptions in wetland ecosystems. Therefore, addressing these invasions is crucial for the preserving and restoring wetlands, necessitating effective and targeted *P. australis* control methods.

1.3 Limitations in current control methods of *Phragmites australis*

Preserving wetlands by controlling invasions of *P. australis* is a major focus of wetland management plans (Lombard et al., 2012; Hazelton et al., 2014). Common methods for managing *P. australis* consist of hydrologic control (flooding), manual removal (cutting, rolling, burning), chemical control (herbicides), or a combination of these approaches (Hazelton et al., 2014).

Hydrologic control involves manipulating water levels to inundate areas invaded by *P. australis* (Rupp et al., 2014), aiming to stress the plant by disrupting the growth cycle, thereby suppressing and reducing its expansion. In some cases, it has been found that water depths greater than 30 cm can inhibit rhizomic spread of *P. australis* and kill any established seedlings (Rupp et al., 2014). However, sustained flooding requires consistent management of water levels, which can be challenging to maintain over extended periods. Additionally, the high tolerance of *P. australis* to water fluctuations and its ability to survive prolonged inundation (Srivastava et al., 2014) make it difficult to control *P. australis* solely through flooding. Extensive aboveground biomass allows *P. australis* to survive high-water levels if enough leaves are in contact with the air to facilitate evapotranspiration (Srivastava et al., 2014). Therefore, flooding can be effective against immature *P. australis* stands, particularly when used in combination with other control methods, such as cutting the aboveground biomass prior to flooding (Hellings & Gallagher, 1992). However, it often proves insufficient against well-established and dense stands where rhizome networks are extensive and resilient, unless coupled with another method such as mowing or herbicides (Hazelton et al., 2014). Additionally, flooding not only affects *P. australis* but also other species within the wetland ecosystem. As the

habitat conditions are altered, stress can be put on native flora and fauna, leading to unintended ecological consequences.

Manual removal techniques include cutting, rolling, and burning of *P. australis* ramets. While more targeted than hydrologic control, these techniques require considerable manual labour, especially where *P. australis* stands are large and dense. Labour requirements often limit large-scale removals due to financial constraints for long-term management efforts (Vyn, 2021). Additionally, manual removal efforts primarily target the above-ground biomass, but do not affect the rhizomes below the ground (Asaeda et al., 2006). Well established *P. australis* invasions have extensive networks of rhizomes, which require considerable damage for long-term suppression. Consequently, even with years of repeated treatment, mechanical removal and hydrologic control methods do not provide sufficient damage to rhizomes, leading to potential regrowth at higher densities (Avers et al., n.d.).

More aggressive approaches such as the use of herbicides are often required for effective long-term suppression of *P. australis*. Broad-spectrum herbicides such as glyphosate- and imazapyr-based herbicides (e.g., Roundup® and Arsenal®) are the most used by wetland managers (Hazelton et al., 2014). These systemic herbicides are absorbed by the plants foliage and translocated through the plant to the underground network of rhizomes (Tu et al., 2001), contributing to high (> 90%) suppression efficacy. Modes of action for these herbicides are through inhibition of 5-enolpyruvylshikimate 3-phosphate (glyphosate; Duke, 1990) and acetolactate (imazapyr; Duke, 1990) synthases, which are responsible for protein synthesis in plants. These enzymes are not present in mammals, reptiles, and fish, making the active ingredient of the herbicides safe for use near wildlife. However, prior to 2019, no glyphosate- or imazapyr-based herbicides were approved for over-water applications due to the presence of

chemical surfactants (e.g. polyethoxylated tallow amine; Mesnage et al., 2019) that are toxic for aquatic species (Tu et al., 2001), severely limiting the ability to control *P. australis* in wetlands and other aquatic habitats. In 2019 approval was received from the Pest Management Regulatory Agency for a new formulation of an imazapyr-based herbicide (Trade name: Habitat® Aqua), in a formulation that is safe for over-water application. The introduction of an approved over-water herbicide has improved previously constrained *P. australis* control efforts in wetland environments. However, the non-selective nature of broad-spectrum herbicides means unintended plant damage can occur if proper care is not taken during application.

1.4 Herbicide application methods for *Phragmites australis* control

Herbicides are typically applied to *P. australis* stands by large machinery (such as amphibious vehicles with a spray boom attached), aerially by helicopters, or manually with backpack sprayers (Tu et al., 2001). The selected application methods depend on the size and density of *P. australis* stands, accessibility of the site to be treated, and presence of sensitive species (Gilbert et al., 2014). Spraying herbicides from amphibious vehicles is a common technique for treating *P. australis* in wetlands. These vehicles are designed to operate on both land and water, providing flexibility across diverse terrains. Large vehicles can treat dense, uniform invasions of *P. australis* in a timely and minimally laborious manner. Although, when *P. australis* is dispersed, heavy machinery can cause unnecessary disturbance to wildlife (Angoh et al., 2021) and leave tracks, destroying habitat along its path.

Aerial spraying via helicopters is the only available application method for sites inaccessible by foot and is extremely efficient where invasions of *P. australis* span large areas (Gilbert et al., 2014). Compared to ground-based methods, aerial application minimizes physical

disturbance to wetland habitats. However, overtreatment from large helicopter swath widths can cause significant collateral damage to surrounding vegetation. In an unpublished analysis, Adam Hogg from Ontario Ministry of Natural Resources and Forestry used RPAS-collected imagery in 2018 to characterize the extent of drift from helicopter application of glyphosate to control *P. australis* in Long Point, Ontario. He found instances of drift up to 90 m beyond the application area and herbicide application exceeded over 40% of the planned spray areas, revealing that non-target effects on adjacent native plants can be significant (Hogg, 2018).

Applying herbicides through backpack spraying allows for increased application precision and is less destructive to surrounding habitats. However, backpack spraying is often impractical for many *P. australis* invasions in wetlands as it is laborious at a large scale, time-consuming, and constrained to sites accessible by foot. This method is best used for smaller or more localized *P. australis* invasions, as it can be highly targeted (Hiremath et al., 2024). The unique distribution of *P. australis* as it grows unconfined in wetlands increases the challenge of precise herbicide application, but high precision of backpack spraying is the only foliar spray method that can treat small or clustered patches of *P. australis* without considerable collateral damage. The localized application of herbicides from backpack spraying therefore reduces the overall quantity of herbicides applied in environments like wetlands and minimizes the impact on non-target species, making it especially useful in areas with mixed vegetation where selective treatment is necessary (DiTomaso & Smith, 2012).

1.5 Current applications of remotely piloted aircraft systems

Remotely piloted aircraft systems (RPASs) represent emerging technology that has dramatically improved environmental applications such as mapping and surveying. This

technology is frequently used to monitor ecosystems and habitats, providing data on vegetation cover (Marcaccio et al., 2015), species distribution (Hrynyk et al., 2024), and habitat changes (Lu & He, 2017). Many studies have used RPAS-collected imagery to classify invasive *P. australis* in wetlands (e.g., Samiappan et al., 2017; Higgs et al., 2021), as mapping the species is a critical component of successful management. One of the major advantages of RPAS-based mapping is the high spatial (sub-meter) and temporal (as desired) resolution compared to satellite images. For example, Brooks et al. (2021) used RPAS-collected imagery to assess aboveground effects of *P. australis* management efforts in the Great Lakes coastal regions. Identifying and monitoring changes in vegetation and land class types becomes much more distinguishable in the high resolution afforded by RPAS-collected images. Further, the flexibility in more frequent data collection allows finer changes to be detected in dynamic ecosystems like wetlands.

In addition to their use in mapping, RPASs have been equipped with spray systems to improve the precision of fertilizer and pesticide applications in agricultural practices (e.g., Faïçal et al., 2014; Giles & Billing, 2015; Hanif et al., 2022). Advancements in RPAS technology have enhanced the efficiency and cost-effectiveness of these applications compared to conventional ground-based (Gibbs et al., 2021) or aerial application methods (Li et al., 2021). Advantages of non-fixed wing RPAS technology for spray applications include their ability to hover at proximity to the target canopy (Martin et al., 2020), rotate in place to change direction, and smaller swath widths compared to aerial applications from airplanes or helicopters (Chen et al., 2021). These factors allow for application areas that are small or irregularly shaped, improving spray precision (e.g., Hunter et al., 2019). Additionally, the use of RPAS technology has significantly reduced labor requirements and enhanced safety for operators as exposure to

hazardous chemicals is minimized and they do not have to enter challenging terrains for applications (Hiremath et al., 2024).

RPASs have the potential to optimize herbicide application for controlling invasive *P. australis*, offering a new balance between effective suppression and non-target vegetation damage. This technology can deliver herbicide with high precision, in combination with the utility of aerial applications, enabling access to otherwise inaccessible area within wetlands. Targeting specific stands of *P. australis* while minimizing exposure to non-target plant species and reducing herbicide drift are particularly beneficial in wetlands, where protecting native vegetation is crucial. Currently, the ability of RPASs to apply herbicides for suppression of *P. australis* is restricted as the safety and precision of this application method that is evidenced in agricultural settings has not been tested in wetlands. Field-based research is required to inform the evaluation by the Pest Management Regulatory Agency before RPAS-based herbicide application to *P. australis* can be permitted in Canada.

1.6 Research objectives

Invasive *Phragmites australis* managers in Canada have limited control options, particularly in areas where on-the-ground access is not possible. While herbicide applications via helicopter have been successful for large-scale invasions (e.g., Robichaud & Rooney, 2021a), the broad treatment areas are not well-suited for small or clustered *P. australis* patches. Our capacity to effectively suppress small patches of *P. australis* in wetlands is essential to control these persistent populations and minimize further spread. While the presence of sensitive species in these habitats necessitates a more precise application method that reduces damage to native wetland vegetation. In this thesis, we aimed to assess RPASs as an additional tool for *P. australis*

management that bridges the gap between available control methods and offers a new solution to the trade-off between effective suppression of *P. australis* and non-target damage to surrounding vegetation.

In the first data chapter, we use field-collected data to assess the initial suppression efficacy of RPAS-based herbicide application and document changes in the plant community in herbicide-treated versus control plots, one year after the herbicide application. In the second data chapter, we used a field-based experiment to document the vertical penetration of herbicide through a *P. australis* canopy when applied by an RPAS and examine the horizontal drift potential away from the application area. We also assess the influence of droplet size on the vertical and horizontal movement of herbicide droplets. In the third data chapter, we use remote-sensing imagery to evaluate the application accuracy of RPAS-based herbicide application and examine the post-treatment effects. In the final thesis chapter, we summarize our findings and report recommendations for RPAS-based herbicide applications to *P. australis* in wetlands.

2.0 Suppression efficacy of RPAS-based herbicide application on invasive *Phragmites australis* in wetlands

2.1 Introduction

An invasive plant, *Phragmites australis* ssp. *australis* has invaded an estimated 13,000 ha of Great Lakes coastal wetland in Ontario alone (Bourgeau-Chavez et al., 2013; Bourgeau-Chavez et al., 2015). Despite extensive wetland management efforts (e.g., those summarized by Hazelton et al., 2014 and Martin & Blossey, 2013), long-term eradication of *P. australis* in many wetlands has not been achieved (Lombard et al., 2012; Martin & Blossey, 2013). As *P. australis* can rapidly colonize when left uncontrolled, and can re-colonize controlled areas when left unmanaged, efforts to suppress invasions have required the integration of more nocuous approaches, such as the use of herbicides.

Broad-spectrum, systemic herbicides (e.g., glyphosate- and imazapyr-based herbicides) are the most widely used chemicals in *P. australis* control (Hazelton et al., 2014) and have achieved high *P. australis* suppression efficacy (> 90% reduction in live biomass) across a range of application times (Knezevic et al., 2013). While glyphosate- and imazapyr-based herbicides (e.g., Roundup® and Arsenal®) are the top herbicide choices for wetland managers, differences in their suppression efficacy on *P. australis* have been studied (Kay, 1995; Back & Holomuzki, 2008; Derr, 2008; Mozdzer et al., 2008). For example, using a pressurized backpack sprayer in 1 m² plots, Mozdzer et al. (2008) reported imazapyr to be 16% more effective (when considering reduction in stem density, canopy height, and percent cover of *P. australis*) than glyphosate. Additionally, the stem density, canopy height, and percent cover of *P. australis* were not only lower in the imazapyr-treated plots in the first year following treatment, but also remained lower

into the second year of follow-up monitoring. Derr (2008) similarly found the reduction in *P. australis* stem density to be 11% greater using an imazapyr-based herbicide when treated in June, and 8% greater when treated in September, compared to a glyphosate-based herbicide in field trials using spray-to-wet application with a pressurized backpack sprayer. Using wipe-on application, Kay (1995) found imazapyr reduced live stems by 75% compared to 33% with the same concentration of glyphosate. While differences in *P. australis* suppression efficacy between glyphosate- and imazapyr-based herbicides have been reported, variations in suppression efficacy based on application method are less studied but provide important context to support wetland management goals (Lombard et al., 2012).

However, herbicide use to suppress *P. australis* presents considerable challenges in conservation efforts, particularly in wetland environments, where application is limited to easily accessible locations. For example, highly precise herbicide application can be achieved with backpack spraying, but this labor-intensive approach is not always feasible when *P. australis* invasions are inaccessible and extensive. While helicopter-based herbicide application can be used to effectively suppress large infestation of *P. australis* even in inaccessible areas, the boom and swath widths used can result in collateral damage to surrounding native plants. Currently, there is no approved herbicide application method that makes it feasible to treat inaccessible, expansive invasions of *P. australis* without extensive off-target effects, rendering long-term suppression of *P. australis* in many wetlands challenging.

Remotely piloted aircraft systems (RPASs) have emerged as a promising tool for pesticide application in the precision agriculture sector, showcasing significant advancements in crop management practices. Studies of RPAS-based pesticide applications have displayed higher efficacy of harvest-aid (characterized by defoliation rate, boll opening rate, and final yield of

cotton plants) than ground-based applications (Cavalaris et al., 2022) and of insecticides (characterized by control of pests on alfalfa) than fixed-wing, pilot-on-board aerial applications (Li et al., 2021). However, existing literature predominantly focuses on RPAS use in agricultural fields where crops present a very uniform height, density, and plant morphology. Little information is available on how this technology performs in natural settings such as wetlands, where the spatial distribution of the target species is non-uniform, and the presence of native species demands precise application. It is unclear whether RPAS-based herbicide applications would provide adequate plant coverage and canopy penetration to suppress *P. australis* in a field setting. Further, it is not well-understood to what extent off-target effects on native plants growing interspersed within *P. australis* or immediately adjacent to it will occur with RPAS applications.

Achieving high suppression efficacy from herbicide treatment is crucial to minimize regrowth of *P. australis* in the following growing seasons. Sub-lethal dosing of herbicide during treatment not only risks re-establishment of *P. australis* networks (Elsay-Quirk & Leck, 2021) but may also lead to herbicide resistance (Government of Ontario, 2001), further increasing the difficulty of long-term suppression efforts. Even when herbicide treatments achieve high levels of suppression (> 90% reduction in live biomass) in the initial treatment, follow-up treatments in the years after initial treatment are typically required (e.g., Kettenring & Adams, 2011; Lombard et al., 2012; Knezevic et al., 2013; Quirion et al., 2018; Zimmerman et al., 2018). Follow-up treatments are often complex as remnant *P. australis* patches that survived the initial herbicide treatment need to be targeted. However, as native vegetation begins to grow back, broadly re-treating across the original *P. australis* patch can lead to unnecessary vegetation damage (Fig. 2-1). Since the cumulative effects of repeated herbicide applications on wetlands are not well-

understood (Crowe et al., 2011), evaluating the efficacy of herbicides and their application method is valuable to minimize the number of reapplications needed, and the amount of herbicide applied in wetlands. For example, glyphosate has been shown to accumulate in soils (Robichaud & Rooney, 2021b), biofilm (Beecraft & Rooney, 2021), litter (Sesin et al., 2022), and groundwater (Crowe et al., 2011). The potential of imazapyr-based herbicides to accumulate in the environment is not as well-studied, but cumulative effects from repeated applications present an equivalent risk (Breckels & Kilgour, 2018).

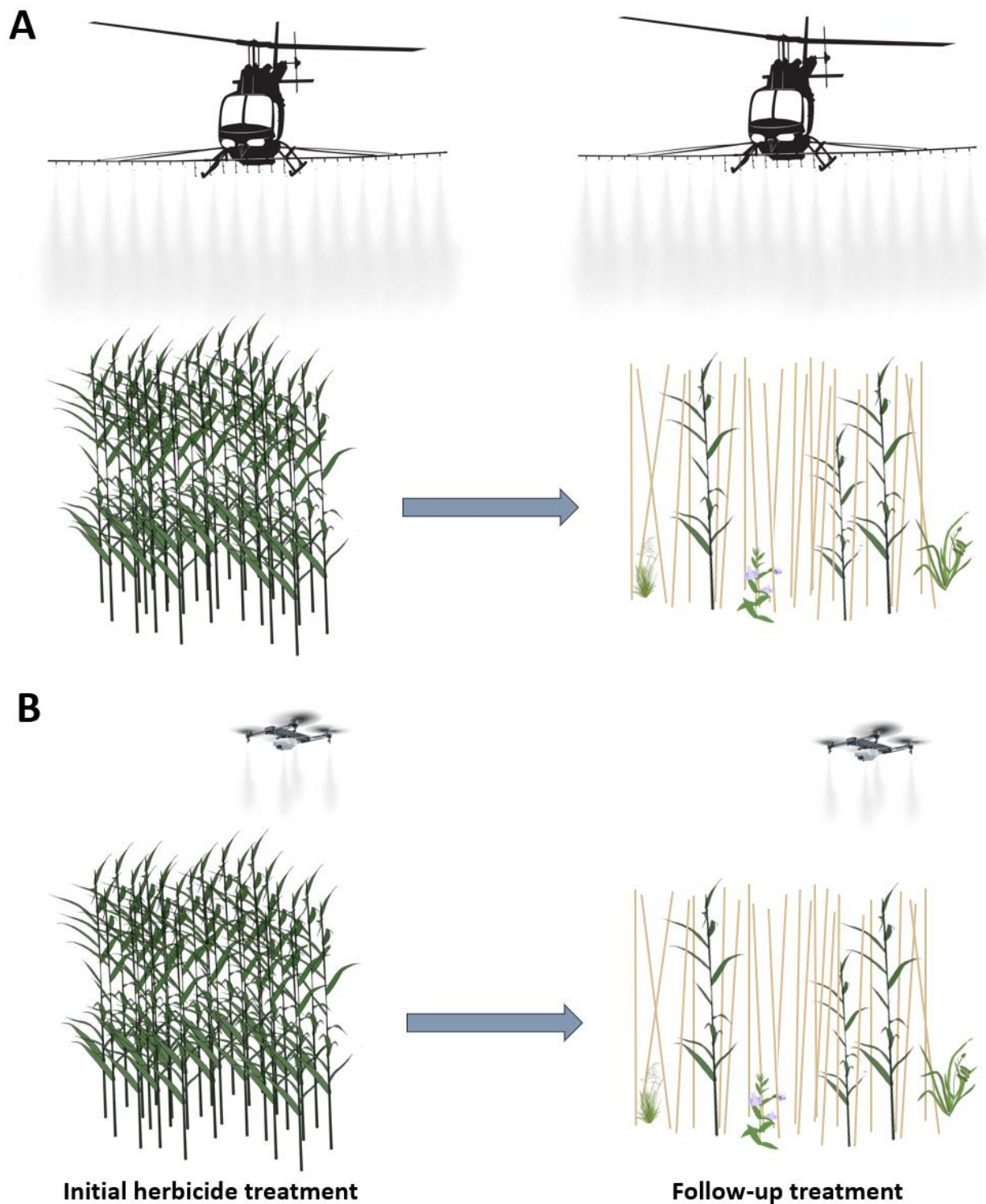


Figure 2-1. Graphic of complexities associated with *Phragmites australis* follow-up treatments after the initial herbicide treatment. The initial herbicide treatment shows a dense monoculture *P. australis* stand that can be effectively treated by either helicopter (A) or RPAS-based (B) herbicide applications. The follow-up treatment shows standing dead as a result of the herbicide treatment, with remnant *P. australis* ramets and native vegetation in close proximity.

To assess the *P. australis* suppression efficacy of RPAS-based herbicide application in provincially significant wetlands in southern Ontario, we conducted the first herbicide application of this kind to control invasive *P. australis* in Canada. Our objectives were: 1) to evaluate the suppression efficacy of RPAS-based herbicide application, quantified as the difference in canopy height and live *P. australis* stem density between treated and adjoining untreated *P. australis* patches and 2) to characterize the extent of native plant recovery one year after herbicide treatment, comparing native plant diversity and community composition in the treated and untreated *P. australis* patches.

2.2 Methods

2.2.1 Study design

A spatially replicated sampling design was used to compare herbicide-treated versus non-treated sample areas, before and after herbicide application, and quantify the effects of the treatment while accounting for spatial and temporal variation.

2.2.2 Field sites

Four Provincially Significant Coastal Wetlands in southern Ontario that were designated as pilot sites for the application of Habitat® Aqua by an RPAS to control invasions of *P. australis* under Research Authorization 0009-RA-22 issued by Health Canada to BASF, the manufacturers of Habitat® Aqua (BASF Canada Inc., Pesticide Registration Number 32374; Proposed Registration Decision PRD2020-17). From these four sites, we selected two wetlands, Baie du Doré and Rondeau Provincial Park (Fig. 2-2), to complete on-the-ground vegetation surveys due to their relative accessibility and dense (> 20 live stems/m²), homogenous *P.*

australis populations. In addition to being listed as Provincially Significant Coastal Wetlands, the ecological values of these wetlands are recognized with environmental designations including as Areas of Natural and Scientific Interest (ANSIs) and Environmentally Sensitive Policy Areas (EPSAs). ANSIs are identified as regions that have significant features containing value worthy of protection, scientific study, or education (Government of Ontario, 2014). EPSAs are designated to protect regions with rare species and valuable ecological functions from nearby development (Environment and Climate Change Canada, 2007).



Figure 2-2. Map of Provincially Significant Wetlands used for on-the-ground vegetation surveys for evaluation of RPAS-based applications of Habitat® Aqua to invasive *Phragmites australis*.

Baie du Doré is a coastal fen situated on the eastern shore of Lake Huron, adjacent to the Bruce Power nuclear power plant and is owned by Ontario Power Generation. This wetland supports significant biodiversity including birds, fish, herpetofauna, and insects (Ball et al., 2003). The *P. australis* invasion at Baie du Doré is characterized by extremely high stem density (> 70 live stems/m²). While management of *P. australis* along Lake Huron's shoreline was proposed in 2013 by the Municipality of Kincardine (Gilbert & Alexander, 2013), Baie du Doré warrants special consideration for RPAS-based herbicide application due to the presence of at-risk turtles (e.g., Spotted turtle and Eastern Spiny Softshell turtle; Ball et al., 2003), which could be injured or killed by heavy machinery or boats.

The Rondeau Provincial Park wetland is part of an extensive swamp-marsh complex located at the southern end of Rondeau Provincial Park in Morpeth, Ontario. The wetland complex supports provincially rare vegetation and serves as critical habitat for migratory marsh birds and a diverse array of species at risk (Ministry of Natural Resources and Forestry, 2015). Additionally, it is a popular tourism destination for outdoor recreation, adding considerable economic value to the region (Ministry of Natural Resources and Forestry, 2015). The specific study area within Rondeau Provincial Park targeted for testing RPAS-based herbicide application is a small swamp slough (~50 ha), surrounded by old growth Carolinian forest. These trees prevent access by amphibious vehicles and the many downed trees and shrubs impede access by backpack sprayers. Furthermore, the narrow width (~50 m) of the slough and the risk of drift and overspray affecting the adjacent trees makes it unsuitable for treatment by large aircraft such as helicopters or planes.

2.2.3 Vegetation surveys

In August 2022, we conducted baseline vegetation surveys at Baie du Doré and Rondeau Provincial Park wetlands prior to the herbicide application. These surveys aimed to assess the initial state of the *P. australis* invasions and determine overall species composition within the sites. We divided each site into control and treatment areas. At Baie du Doré, we selected two *P. australis* patches of comparable size (~ 0.03 ha) and live stem density (~ 75 live stems/m²) for surveying. The northernmost patch served as the control, while the southernmost patch was designated for treatment (Fig. 2-3). At Rondeau Provincial Park, we divided the slough medially from north-east to south-west with the eastern side of the slough serving as the control and the western side designated for herbicide treatment (Fig. 2-4).

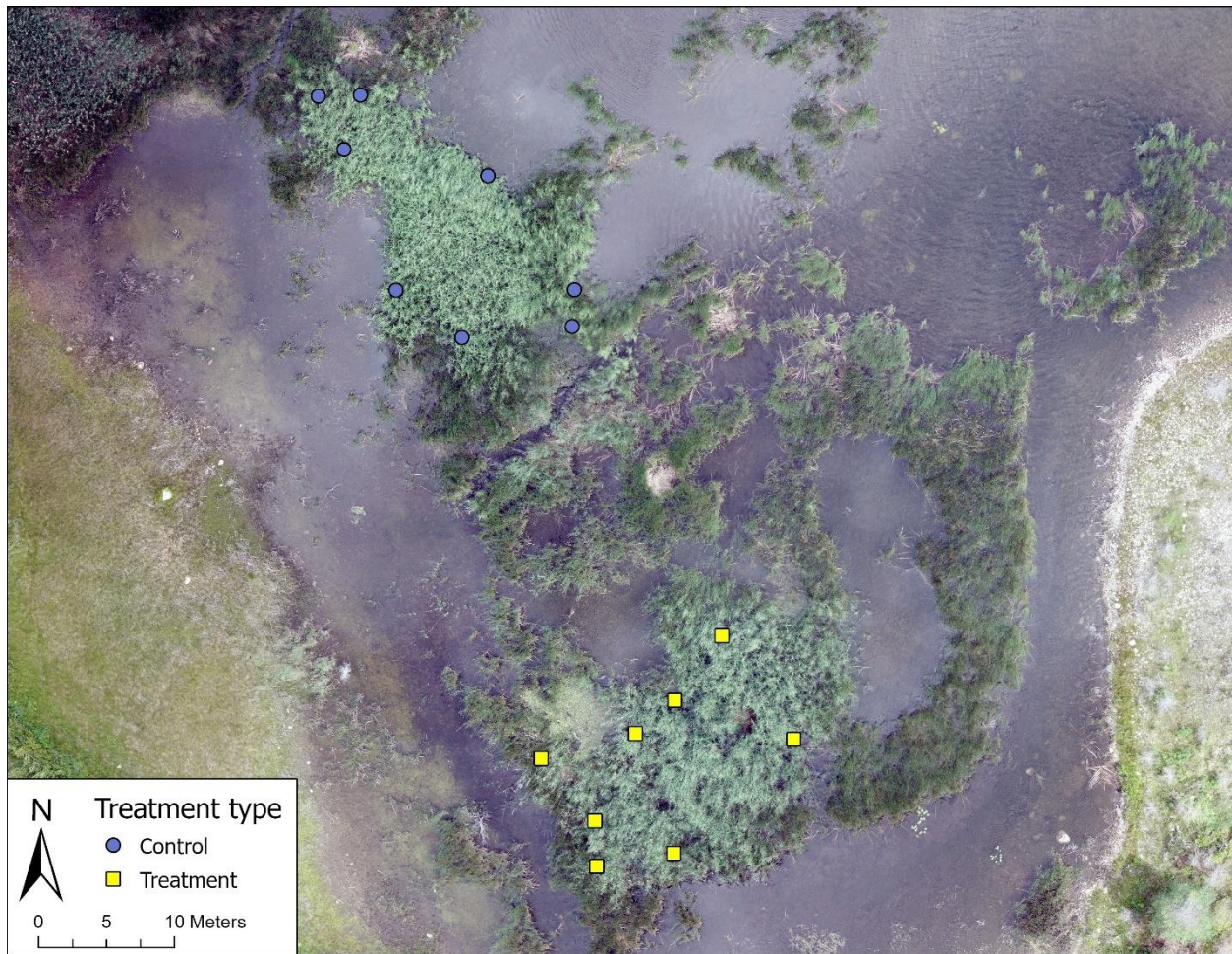


Figure 2-3. Locations of experimental control (n=8) and treatment (n=8) plots for RPAS-based herbicide treatment in Baie du Doré wetland. The northern patch was left as the control (untreated) and the southern patch was designated as the treatment side to be sprayed with Habitat® Aqua by an RPAS. Purple circles represent control plots and yellow squares represent treatment plots. Basemap imagery was collected by Ontario Power Generation's Advanced Inspection and Maintenance team in 2022.

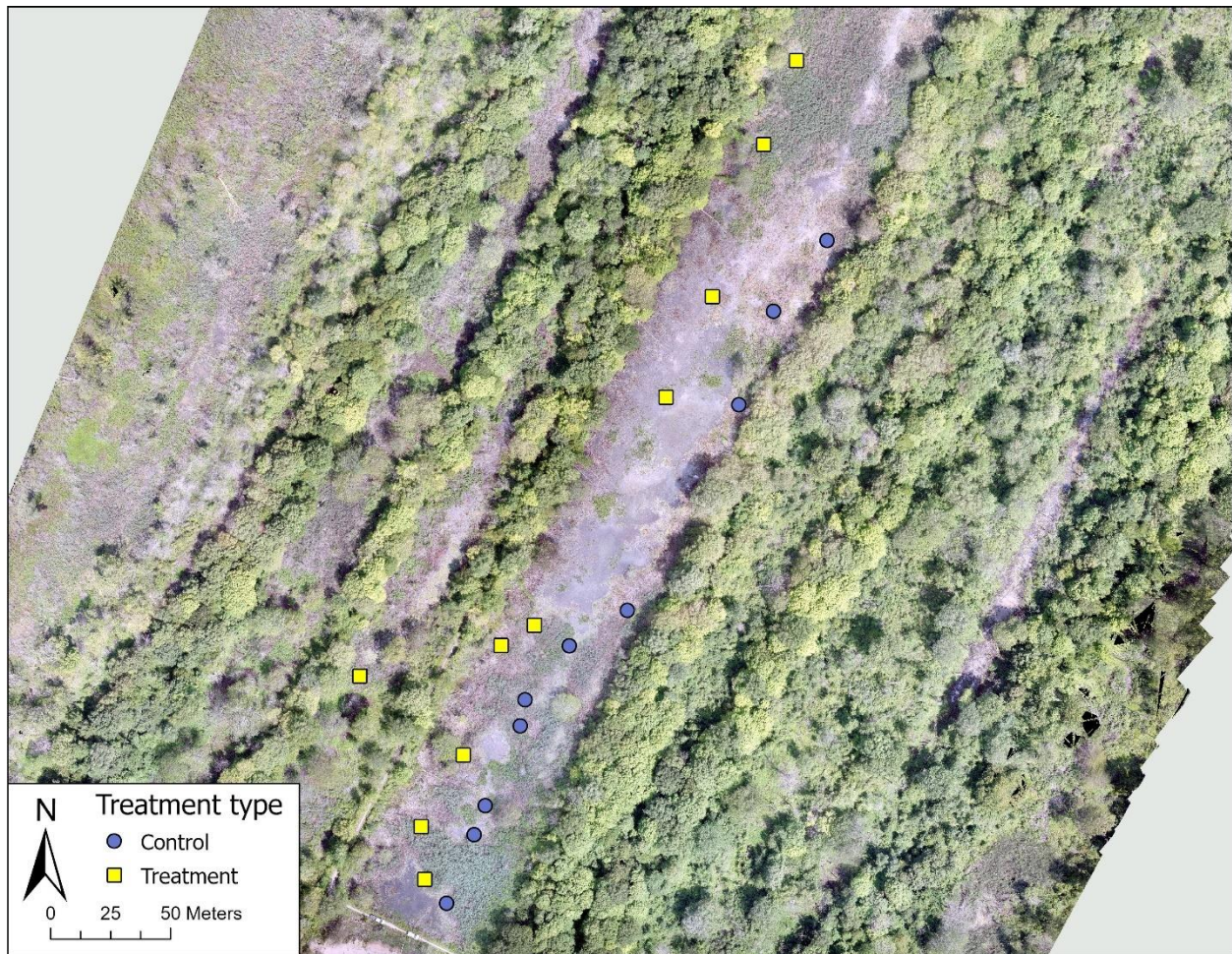


Figure 2-4. Locations of experimental control (n=10) and treatment (n=10) plots for RPAS-based herbicide treatment in Rondeau Provincial Park wetland. The east side of the slough was left as the control (untreated) side and the west side of the slough was designated as the treatment side to be sprayed with Habitat® Aqua by an RPAS. Purple circles represent control plots and yellow squares represent treatment plots. Basemap imagery was collected by Ontario Power Generation’s Advanced Inspection and Maintenance team in 2022.

The summer before herbicide application, within each control and proposed treatment area, we completed quadrat surveys by deploying 1 m² survey plots (quadrats; N=16 at Baie du Doré, Fig. 2-3; N=20 at Rondeau Park, Fig. 2-4) within dense (> 20 live stems/m²) *P. australis* patches. Half of the plots were established in the control areas, and the other half in the treatment areas. We paired control and treatment plots by water depth measured in the center of the quadrat to ensure we captured a similar range of water depths (and hence of vegetation community composition) in the control and treatment areas before the herbicide application. Water depth of

the plots ranged from 0 to 43 cm. By comparing control and treatment plots, both before and after herbicide treatment, we can account for year over year variation in vegetation communities that is not attributable to the herbicide treatment.

During the pre- and post-treatment vegetation surveys of each plot, we measured water depth, density of live and dead *P. australis* stems, and plant-canopy height. Vascular plants rooted in the plots were identified to species, wherever possible, following the *Field Manual of Michigan Flora* (Voss & Reznicek, 2013) and relative cover of each plant was estimated to document the current vegetation community present in each plot. Additionally, we recorded GPS coordinates with sub-meter accuracy (SXPro GNSS, Geneq Inc., Montreal, QC, Canada) at the center of each plot and placed bamboo stakes with flagged labels at each corner of the plot to facilitate finding plot location and orientation the following year. In August 2023, we conducted follow-up vegetation surveys at both wetlands following the same sampling methods to assess the post-treatment state of the plots.

2.2.4 RPAS-based herbicide application

In September 2022, imazapyr as an isopropylamine salt (concentration 240 g/L), under the tradename Habitat® Aqua (BASF Canada Inc., Pesticide Registration Number 32374; Proposed Registration Decision PRD2020-17), was applied to the designated treatment areas by an RPAS under the Research Authorization 0009-RA-22. The herbicide was applied at a rate of 4.68L/ha and total spray volume of 100L/ha (includes 0.25% v/v Aquasurf® non-ionic spray adjuvant; Norac Concepts Inc., Pesticide Registration Number 32152), by a licensed drone pilot and pesticide applicator (L-208-8129866762). The RPAS used for application was a quadcopter (Hylio Inc., Texas, USA), equipped with eight AIXR110015 spray nozzles (two per rotor) and

sprayed with a medium droplet size (236-340 µm; American Society of Agricultural and Biological Engineers, n.d.).

Application procedures adhered to Habitat® Aqua’s label recommendations for reducing spray drift and weed specific instructions for *P. australis* (BASF Canada Inc., n.d.). In the field, treatment areas were delineated either with ground control points by flying the RPAS above the patch and confirming the boundaries by radio communication to the pilot, or by visual observation of *P. australis* through the first person-view camera by the pilot. Visual observations in the field showed no evidence of spray drift due to wind gusts as the rotors of the RPAS primarily pushed the herbicide droplets downwards and wind speed was very low (Table 2-1). We observed *P. australis* leaves to be dry within minutes of the herbicide application. Secondary treatment such as burning, rolling, or cutting of standing dead biomass was not undertaken after herbicide application.

Table 2-1. Treatment dates and weather parameters during the RPAS-based herbicide application at two pilot sites used for on-the-ground vegetation surveys. Temperature and relative humidity were measured with an Extech RH300 hygro-thermometer (Extech Instruments, Waltham, MA, USA). Wind direction and wind velocity were measured with a Kestrel 1000 anemometer (Kestrel Instruments, Boothwyn, PA, USA).

Site	Date of treatment	Temperature (°C)	Relative humidity (%)	Wind direction	Wind velocity (kph)
Baie du Doré	September 1, 2022	25.0	53.6	West	1.7
Rondeau Provincial Park	September 14, 2022	23.0	63.4	South	5.0

2.2.5 Statistical methods

To test for a significant effect ($\alpha = 0.05$) from the RPAS-based herbicide treatment on the suppression efficacy (live *P. australis* stem density, total *P. australis* stem density, and

canopy height) and plant diversity response variables (species richness (S), Shannon-Weiner diversity (H'), Simpson's reciprocal diversity index ($1/D$), Pielou's evenness (J), and mean coefficient of conservatism (mean CC_s)), we used a Kruskal-Wallis test (Kruskal & Wallis, 1952). Since the response variables violated the assumptions of a one- or two-way ANOVA (normality of the residuals, that could not be corrected by transformation, and homogeneity of variance of the residuals), we opted for the nonparametric equivalent that does not hold the same assumptions about the distribution of residuals. If the null hypothesis was rejected by the Kruskal-Wallis test, we used a Dunn's test for multiple planned comparisons of median differences (Dunn, 1964) with a Bonferroni correction to adjust the p -value in accordance with the number of pairwise comparisons. All statistical analyses were conducted in R v. 4.3.2 (R Core Team, 2023).

Coefficients of conservatism are values assigned to plant species by expert botanists, ranging from 0 to 10. These values represent the ability of a given plant species to tolerate disturbance, with low values representing exotic species and high values representing plant species of high conservation value. Coefficients of conservatism for each identified plant species were obtained from *Floristic Quality Assessment System for Southern Ontario* (Oldham et al., 1995). Mean coefficients of conservatism were calculated following the recommended equation for CC_s by Kutcher and Forrester (2018). Shannon-Weiner diversity and Simpson's reciprocal diversity indices were calculated with the R package "vegan" (Oksanen et al., 2013). Table 2-2 provides details of the equations used to calculate all plant diversity metrics.

Table 2-2. Equations and associated references used to calculate plant diversity metrics. The variable s represents a given species identified in the quadrat and p_i represents the proportion of a given species in the quadrat.

Plant diversity metric	Equation	Reference
Species richness	$S = \sum s$	Fisher et al., 1943
Pielou's evenness	$J' = \frac{H'}{H_{max}} \quad H_{max} = \ln S$	Pielou, 1975
Simpson's reciprocal diversity	$\frac{1}{D} = \frac{1}{\sum p_i^2}$	Hill, 1973
Shannon-Weiner diversity	$H' = -\sum (p_i * \ln(p_i))$	Shannon, 1948
Mean coefficient of conservatism	$weighted\ mean\ CC_s = \frac{\sum CC_i * p_i}{\sum p_i}$	Kutcher & Forrester, 2018

2.3 Results

We assessed suppression efficacy of RPAS-based herbicide application on *P. australis* in wetlands through response variables including live *P. australis* stem density and canopy height. Live *P. australis* stem density and canopy height reduced dramatically one year after the herbicide treatment of Habitat® Aqua with the RPAS (Fig. 2-5). Specifically, live *P. australis* stem density in the herbicide-treated plots decreased by > 99% across both sites (Kruskal-Wallis: $H_3 = 40.6$, $p < 0.001$), while total *P. australis* stem density (including standing dead stems) did not change significantly after the herbicide treatment (Kruskal-Wallis: $H_3 = 0.44$, $p = 0.931$). Median canopy height did not differ among 2022 control, 2022 treatment, and 2023 control plots, but did decrease significantly by ~94% in the herbicide-treated plots, (Kruskal-Wallis: $H_3 = 40.4$, $p < 0.001$). We used a Dunn's post-hoc test for planned comparisons to support our interpretation of the Kruskal-Wallis results. These planned comparisons contrast the treatment plots before and after herbicide application as well as the 2023 (post-) treatment and control plots to understand the nature of any significant effect of treatment; secondly, contrast the control plots before and

after to assess any interannual differences; and thirdly, contrast the 2022 (pre-) treatment and control plots to evaluate the pre-treatment similarity between treatment and controls. Results from the post-hoc Dunn's test for live *P. australis* stem density and canopy height are summarized in Table 2-3.

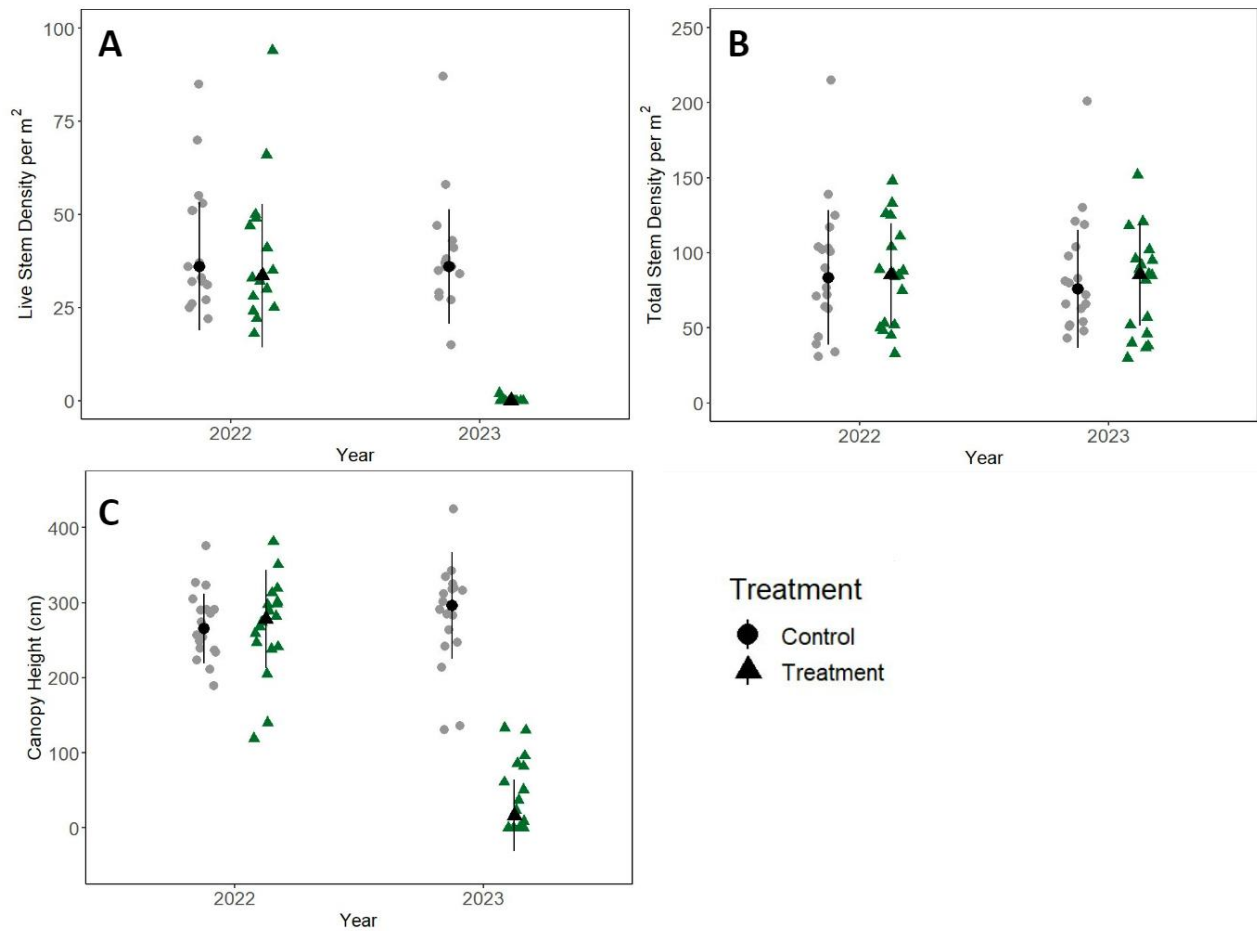


Figure 2-5. Jitter plot comparison of live *Phragmites australis* stem density (A), total *P. australis* stem density (B), and canopy height (C) between control and RPAS-based herbicide treated plots, pre-treatment (2022), and post-treatment (2023). Note that total stem density includes both live and dead stems of *P. australis*. Grey circles represent control sites, and green triangles represent treated sites. Black symbols represent the median value, and error bars represent standard deviation. Created with ggplot2 (Wickham, 2016).

Table 2-3. Dunn’s test results for live *Phragmites australis* stem density and canopy height for control and RPAS-based herbicide treated plots, pre-treatment (2022), and post-treatment (2023). Columns “n1” and “n2” refer to sample size of plots for Group 1 and Group 2, respectively. Adjusted *p*-value is based on the Bonferroni correction method for multiple planned comparisons.

Response variable	Group 1	Group 2	n1	n2	<i>p</i> value	Adjusted <i>p</i> value
Live <i>P. australis</i> stem density	2022 Control	2023 Control	18	18	0.917	1
	2022 Control	2022 Treatment	18	18	0.832	1
	2023 Control	2023 Treatment	18	18	<0.001	<0.001
	2022 Treatment	2023 Treatment	18	18	<0.001	<0.001
Canopy height	2022 Control	2023 Control	18	18	0.366	1
	2022 Control	2022 Treatment	18	18	0.836	1
	2023 Control	2023 Treatment	18	18	<0.001	<0.001
	2022 Treatment	2023 Treatment	18	18	<0.001	<0.001

The RPAS-based herbicide treatment had a substantial effect on response variables related to plant diversity (species richness, Shannon-Weiner diversity, Simpson’s reciprocal diversity index, Pielou’s evenness, and mean coefficient of conservatism), one year after the herbicide application (Fig. 2-6). No difference in species richness was observed among the 2022 control, 2022 treatment, and 2023 control plots, with a common median value of ~4-5 species per plot. However, median species richness in the herbicide treated plots decreased significantly by approximately 33% (Kruskal-Wallis: $H_3 = 18.8$, $p < 0.001$). Similarly, the median value for the Shannon-Weiner diversity index decreased by nearly 73% in the treated plots after herbicide application (Kruskal-Wallis: $H_3 = 23.7$, $p < 0.001$). The median value for the Simpson’s reciprocal diversity index was not different among the 2022 control, 2022 treatment, and 2023 control plots with median values ~2 in the untreated plots, while a reduction of 50% to values ~1 in the 2023 treated plots, was observed (Kruskal-Wallis: $H_3 = 23.1$, $p < 0.001$). The median value for Pielou’s evenness decreased by 73% in herbicide treated plots compared to 2022 treatment plots (Kruskal-Wallis: $H_3 = 18.3$, $p < 0.001$) whereas evenness did not significantly differ among 2022

control, 2022 treatment, and 2023 control plots. The Kruskal-Wallis test revealed there was a significant difference among the four groups ($H_3 = 8.73$, $p = 0.033$) for the mean coefficient of conservatism. However, from the post-hoc Dunn's test, no significant differences were observed among groups due to the strictness of the Bonferroni correction used for planned comparisons. Planned comparisons from the post-hoc Dunn's test for all plant diversity response variables are summarized in Table 2-4.

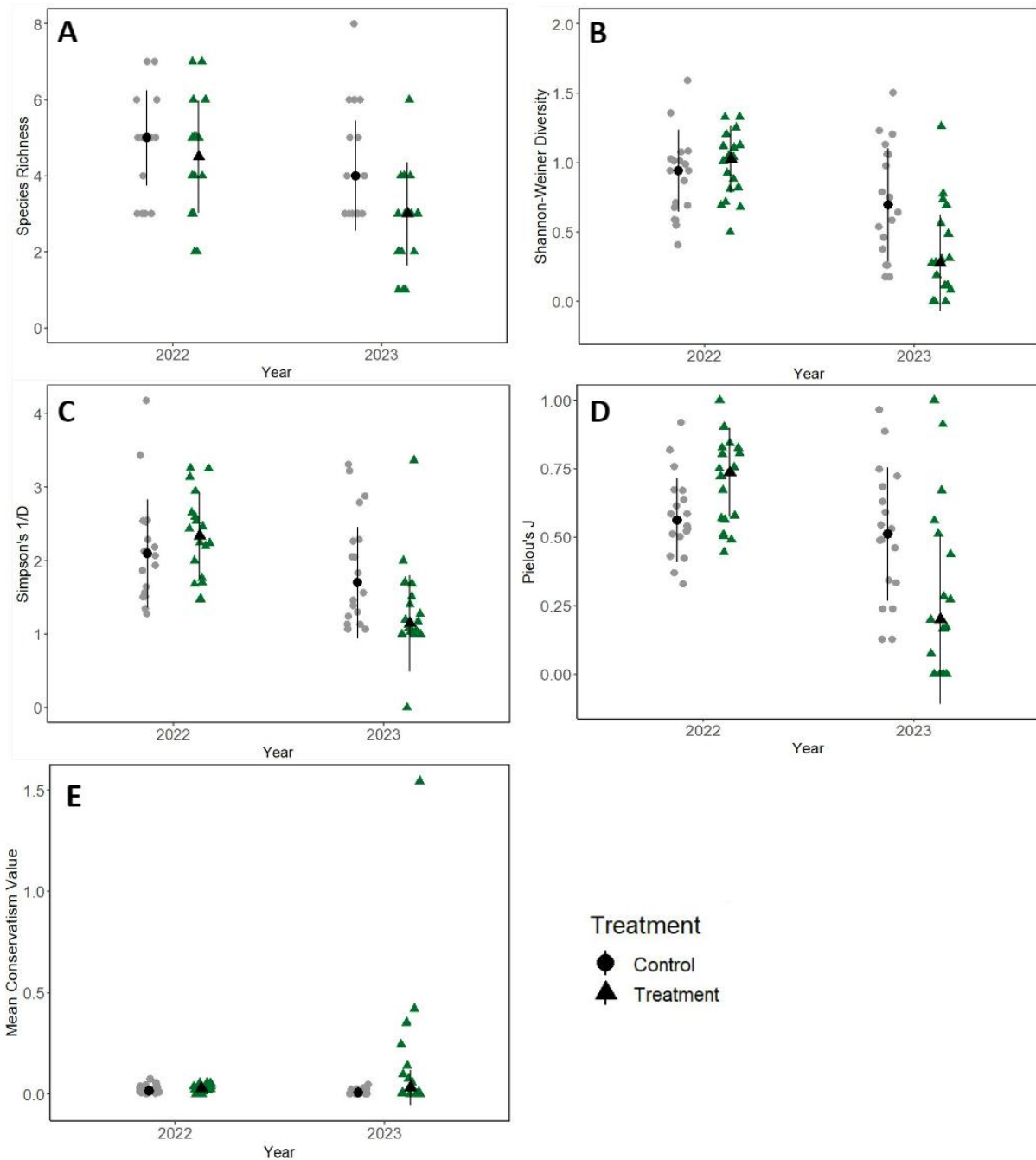


Figure 2-6. Jitter plot comparison of species richness (S ; A), Shannon-Weiner diversity (H' ; B), Simpson's reciprocal diversity index ($1/D$; C), Pielou's evenness (J ; D), and mean coefficient of conservatism value (CC_s ; E) between control and RPAS-based herbicide treated plots, pre-treatment (2022), and post-treatment (2023). Grey circles represent control sites, and green triangles represent treated sites. Black symbols represent the median value, and error bars represent standard deviation. Created with ggplot2 (Wickham, 2016).

Table 2-4. Dunn’s test results for species richness (S), Shannon-Weiner diversity (H'), Simpson’s reciprocal diversity index ($1/D$), Pielou’s evenness (J), mean coefficient of conservatism value (CC_s) for control and RPAS-based herbicide treated plots, pre-treatment (2022), and post-treatment (2023). Adjusted p value is based on the Bonferroni correction method for multiple planned comparisons.

Response variable	Group 1	Group 2	n1	n2	p value	Adjusted p value
Species richness	2022 Control	2023 Control	18	18	0.190	0.760
	2022 Control	2022 Treatment	18	18	0.485	1
	2023 Control	2023 Treatment	18	18	0.006	0.025
	2022 Treatment	2023 Treatment	18	18	<0.001	0.003
Shannon-Weiner diversity	2022 Control	2023 Control	18	18	0.297	1
	2022 Control	2022 Treatment	18	18	0.381	1
	2023 Control	2023 Treatment	18	18	<0.001	0.031
	2022 Treatment	2023 Treatment	18	18	<0.001	<0.001
Simpson’s reciprocal diversity index	2022 Control	2023 Control	18	18	0.250	1
	2022 Control	2022 Treatment	18	18	0.379	1
	2023 Control	2023 Treatment	18	18	0.013	0.050
	2022 Treatment	2023 Treatment	18	18	<0.001	<0.001
Pielou’s evenness	2022 Control	2023 Control	18	18	0.435	1
	2022 Control	2022 Treatment	18	18	0.136	0.544
	2023 Control	2023 Treatment	18	18	0.055	0.220
	2022 Treatment	2023 Treatment	18	18	<0.001	<0.001
Mean conservatism value	2022 Control	2023 Control	18	18	0.078	0.314
	2022 Control	2022 Treatment	18	18	0.304	1
	2023 Control	2023 Treatment	18	18	0.025	0.101
	2022 Treatment	2023 Treatment	18	18	0.583	1

At Baie du Doré, a total of eight species were identified in the plots in 2022, while nine species were identified in 2023. At Rondeau Provincial Park, 15 species were identified in the plots in 2022, and 17 species were identified in 2023. Table 2-5 details all vascular plant species identified in the survey plots at Baie du Doré and Rondeau Provincial Park wetlands. At Baie du Doré, only two plant species persisted in the herbicide-treated plots (*Lemna minor* and *P. australis*) one year after the initial herbicide treatment. After the RPAS-based herbicide treatment, five species including *Typha x glauca* were excluded from the herbicide-treated plots and seven species that were not found in the baseline vegetation surveys appeared in the

herbicide-treated plots, including the native *Impatiens capensis*. At Rondeau Provincial Park, four plant species persisted in the herbicide-treated plots (*Bidens tripartita*, *Cicuta bulbifera*, *Hydrocharis morsus-ranae*, and *Lemna minor*) in the year following the initial herbicide treatment. After the RPAS-based herbicide treatment, six species including *P. australis* were excluded from the herbicide-treated plots and three species that were not found in the baseline vegetation surveys appeared in the herbicide-treated plots, including the native *Zizania aquatica*. At Baie du Doré the native plant cover decreased by 15% in the control plots, while increasing by 20% in the herbicide-treated plots. While at Rondeau Provincial Park, native plant cover decreased by 16% in the control plots and by 65% in the herbicide-treated plots. In contrast, the non-native plant cover at Baie du Doré increased by 50% in the control plots and decreased by 100% in the herbicide-treated plots. At Rondeau Provincial Park, non-native plant cover similarly increased by 55% in control plots and decreased by 25% in the herbicide treated plots.

Table 2-5. List of vegetation species identified in experimental control and treatment plots at Baie du Doré and Rondeau Provincial Park wetlands in the year before (2022) and year after (2023) RPAS-based herbicide treatment to *Phragmites australis*.

Site	Year	Treatment type	Species
Baie du Doré	2022	Control	<i>Elodea canadensis</i>
			<i>Lemna minor</i>
			<i>Myriophyllum sibiricum</i>
			<i>Najas flexilis</i>
			<i>Phragmites australis</i>
			<i>Potamogeton natans</i>
			<i>Typha x glauca</i>
	2022	Treatment	<i>Lemna minor</i>
			<i>Lycopus americanus</i>
			<i>Myriophyllum sibiricum</i>
			<i>Najas flexilis</i>
			<i>Phragmites australis</i>
	2023	Control	<i>Potamogeton zosteriformis</i>
			<i>Typha x glauca</i>
			<i>Ceratophyllum demersum</i>
			<i>Geranium robertianum</i>
			<i>Impatiens capensis</i>
			<i>Lemna minor</i>

			<i>Phragmites australis</i> <i>Typha x glauca</i>
Rondeau Provincial Park	2023	Treatment	<i>Carex</i> spp. <i>Ceratophyllum demersum</i> <i>Erechtites</i> spp. <i>Impatiens capensis</i> <i>Lemna minor</i> <i>Lemna trisulca</i> <i>Persicaria lapathifolia</i> <i>Phragmites australis</i> <i>Utricularia vulgaris</i>
	2022	Control	<i>Bidens tripartita</i> <i>Boehmeria cylindrica</i> <i>Carex</i> spp. <i>Cephalanthus occidentalis</i> <i>Cicuta bulbifera</i> <i>Galium labradoricum</i> <i>Hydrocharis morsus-ranae</i> <i>Impatiens capensis</i> <i>Lemna minor</i> <i>Persicaria amphibia</i> <i>Phragmites australis</i> <i>Prunella vulgaris</i> <i>Thelypteris palustris</i>
	2022	Treatment	<i>Bidens tripartita</i> <i>Cephalanthus occidentalis</i> <i>Cicuta bulbifera</i> <i>Circaea lutetiana</i> <i>Cornus sericea</i> <i>Hydrocharis morsus-ranae</i> <i>Lemna minor</i> <i>Lemna trisulca</i> <i>Persicaria amphibia</i> <i>Phragmites australis</i>
	2023	Control	<i>Bidens tripartita</i> <i>Boehmeria cylindrica</i> <i>Carex</i> spp. <i>Cephalanthus occidentalis</i> <i>Cicuta bulbifera</i> <i>Circaea lutetiana</i> <i>Hydrocharis morsus-ranae</i> <i>Impatiens capensis</i> <i>Leersia oryzoides</i> <i>Lemna minor</i> <i>Lemna trisulca</i> <i>Mimulus ringens</i> <i>Persicaria amphibia</i> <i>Persicaria lapathifolia</i> <i>Phragmites australis</i> <i>Sium suave</i>

2023	Treatment	<i>Sparganium eurycarpum</i>
		<i>Bidens tripartita</i>
		<i>Cicuta bulbifera</i>
		<i>Hydrocharis morsus-ranae</i>
		<i>Leersia oryzoides</i>
		<i>Lemna minor</i>
		<i>Sparganium eurycarpum</i>
		<i>Zizania aquatica</i>

2.4 Discussion

While there is established literature on both the suppression efficacy of imazapyr for *P. australis* (e.g., Mozdzer et al., 2008; Whyte et al., 2009; Knezevic et al., 2013) and the use of RPAS for precision pesticide applications in agricultural contexts (e.g., Li et al., 2021; Cavalaris et al., 2022), this is the first study to report on RPAS-based herbicide application for *P. australis* suppression in wetlands. We aimed to evaluate the extent of *P. australis* suppression and the response of the native plant community to the herbicide application in the first year after treatment. Our suppression efficacy in terms of the reduction in live *P. australis* stem density was as high or higher than any imazapyr-based *P. australis* suppression study reported in the literature (Mozdzer et al., 2008; Whyte et al., 2009; Knezevic et al., 2013), at 99% reduction. We also observed ~94% reduction in plant canopy height, although the lack of secondary treatment (e.g., mowing or rolling) left many standing dead *P. australis* stems. In terms of native plant community recovery in the year following herbicide application, we observed a small but significant reduction in species richness, evenness, and diversity which was in line with our expectations from published studies (Zimmerman et al., 2018; Robichaud & Rooney, 2021a; Jordan, 2022),.

Initial suppression of *P. australis* from the RPAS-based herbicide application was successful within the treatment areas at Baie du Doré and Rondeau Provincial Park wetlands. Live *P. australis* stem density significantly decreased in RPAS herbicide-treated plots compared to control plots. The reduction of live stem density was over 99%, with only one of the 18 treatment plots surveyed after the RPAS-based herbicide treatment having live *P. australis* stems. Further, canopy height reduced by ~94% in the RPAS-based herbicide-treated plots. Our results mirror findings from Knezevic et al. (2013) who found imazapyr applied at a rate of 560 g a ha⁻¹ from a backpack sprayer provided nearly 100% control of *P. australis* in their plots, with only one live stem per meter squared observed one year after herbicide application. Mozdzer et al. (2008), also using backpack spraying, report over 95% reduction in *P. australis* (considering live stem density, canopy height, and percent cover in 10 m² macro plots) when treated with a 5% imazapyr concentration. When Habitat® Aqua was applied by helicopter with a 7% solution, Whyte et al. (2009) found that *P. australis* abundance was reduced by ~90%. Our study using RPAS-based imazapyr application met or exceeded the efficacy reported in these other studies. These results support RPAS-based herbicide application as an equally effective application method for initial suppression of *P. australis* in wetlands.

In addition to the impact on *P. australis* abundance, there was also an anticipated significant reduction in species richness, evenness, and diversity in the year following the RPAS-based herbicide application. An initial reduction of these response variables has been similarly reported (e.g., Zimmerman et al., 2018; Robichaud & Rooney, 2021a; Jordan, 2022). In Robichaud and Rooney's (2021a) study, 1 m² plots that were helicopter treated with glyphosate in Long Point and Rondeau Provincial Parks showed a 33% reduction in species richness, 69% reduction in Shannon-Weiner diversity, 9% reduction in Simpson's reciprocal diversity index,

and 63% reduction in Pielou's evenness one year after the initial herbicide treatment, exhibiting similar values to our plots that had reductions of 33%, 73%, 50%, and 73%, respectively. Jordan (2022) continued monitoring Robichaud and Rooney's (2021a) plots for up to five years after herbicide was applied. She concluded that a minimum of three years after the initial herbicide treatment will be required for the plant community to begin transitioning to predominantly native vegetation (Jordan, 2022). Although, the level of hydrologic disturbance and health of the seedbank will largely dictate whether native plants will re-establish after herbicide suppression of *P. australis* (Rohal et al., 2019) and the plots surveyed in our studies represent biodiverse coastal wetlands, likely representing a best-case scenario for plant community recovery from a strong seedbank. While we expect the response variables in our plots to follow a similar plant recovery trajectory, continuous monitoring of the herbicide-managed wetlands is needed document the short- and long-term plant community composition changes (Kettenring & Adams, 2011; Bonello & Judd, 2020; Robichaud & Rooney, 2021a). No secondary treatment (e.g., burning, rolling, or cutting of standing dead biomass) was completed at either of our study sites. The presence of standing dead biomass in the first year after treatment likely increased shading in the surveyed plots and may have hindered germination of species from the seedbank that rely on high light interception through the canopy (Minchinton et al., 2006). Although, Robichaud and Rooney (2021a) found that standing dead biomass and light penetration of areas that did not undergo secondary treatment reached similar levels to areas that underwent burning, rolling, or cutting within a two-year timeframe. Therefore, in the long-term, the presence of standing dead biomass should not contribute to reduced species richness, evenness, and diversity. Additionally, inclusion of secondary treatment can offset the benefits of RPAS-based herbicide application,

since heavy machinery is needed to mechanically knock down the standing dead biomass and risks crushing sensitive species in its path (Angoh et al., 2021).

Although the mean coefficient of conservatism remained relatively low, with the highest observed mean CC_s value being 1.5 of a possible 10, the increase in value from the control plots suggests that *P. australis* ($CC_s = 0$) is no longer the dominant species in the plots. After the herbicide treatment, *Hydrocharis morsus-ranae* (European Frog-bit; $CC_s = 0$) became the dominant species in the treated plots at Rondeau Provincial Park. Secondary invasion by species like *H. morsus-ranae* have been documented previously (e.g., Bonello & Judd, 2020; Robichaud & Rooney, 2021a), as the suppression of *P. australis* without active planting or reseeding creates an open niche for secondary invaders to establish within (Kettenring & Tarsa, 2020). For example, Robichaud and Rooney (2021a) monitoring the effects of herbicide treatment in Rondeau Provincial Park also observed secondary invasion by *H. morsus-ranae* in the first years after *P. australis* suppression. However, at Baie du Doré a secondary invasive species did not establish in the plots, instead the annual *Impatiens capensis* (Spotted Jewelweed; $CC_s = 4$) became most abundant. The observed difference between the two sites may be due to the pre-treatment plant community composition (Pearson et al., 2016). For example, *H. morsus-ranae* was observed in nearly all pre-treatment plots at Rondeau Provincial Park but not in the plots from Baie du Doré. This suggests that pre-treatment surveys may help predict where secondary invasions are likely to occur following *P. australis* suppression and could be useful in generating guidance on where seeding or planting could be most valuable to facilitate native plant community recovery. Additionally, the increase in water depths at Rondeau Provincial Park between 2022 (19 cm; std. 13 cm) and 2023 (32 cm; std. 11 cm) was likely a contributing factor. Because *H. morsus-ranae* is a floating macrophyte, these deeper water conditions likely favored

its establishment in the absence of *P. australis*. Unlike Rondeau Provincial Park, water depths at Baie du Doré decreased between 2022 (23 cm; std. 10 cm) and 2023 (13 cm; std. 9 cm), resulting in moist conditions which could favor germination of annuals like *I. capensis*. Interestingly, *I. capensis* was not present in the 2022 pre-treatment plots at Baie du Doré, although we would not expect it to coexist with the dense *P. australis* targeted for treatment. Instead, *I. capensis* likely either dispersed into the treated area following herbicide application (e.g., its seeds can travel up to 2 m through ballistic dispersal; Hayashi et al., 2009). Alternatively, it may have been waiting in the seedbank for suitable conditions to arise, as *I. capensis* seeds can remain viable in the seedbank for up to three years (Perglová et al., 2009).

Additional research in lower density *P. australis* patches (< 20 live stems/m²) or various spatial distributions of *P. australis* patches with interspersed vegetation would help to justify broader generalizability of our results which were collected from relatively contiguous patches with high live *P. australis* stem density (> 20 live stems/m²). Our main goal was to evaluate the initial suppression efficacy of RPAS-based herbicide application to *P. australis* in wetlands. Long-term monitoring is necessary to assess the outcome of *P. australis* suppression for the vegetation community (Jordan 2022); although notably a review found that 40% of studies on *P. australis* management lasted only one year (Kettenring & Adams, 2011; Hazelton et al., 2014). Based on existing *P. australis* management literature using systemic herbicides (Derr, 2008; Mozdzer et al., 2008; Knezevic et al., 2013), and the reported trajectory of vegetation recovery (Bonello & Judd, 2020; Robichaud & Rooney, 2021a), we anticipate that the RPAS-treated sites will follow a similar succession, provided adequate follow-up treatments and monitoring take place (Lombard et al., 2012). However, while the dominance of *H. morsus-ranae* at Rondeau Provincial Park and *I. capensis* at Baie du Doré may be due to hydrologic disturbance, the health

of the seedbank, or sources of native plant propagules (Rohal et al., 2019), the differences in these biodiverse coastal wetlands in the first year after herbicide treatment highlight the need for further examination of the complex factors involved in plant recovery following *P. australis* suppression in dynamic wetland environments. Further research is needed to evaluate the broader implications of RPAS-based herbicide applications at a larger extent than was feasible under our Research Authorization (limited to 2 ha of intended treatment area per site) and continued monitoring should be implemented to assess native plant recovery.

Our results have important implications for wetland management and conservation efforts, particularly in the context of invasive species control and habitat restoration. The substantial reduction in *P. australis* live stem density and canopy height following RPAS-based herbicide application represents successful initial *P. australis* suppression, but the widely reported need for follow-up treatments to maintain long-term suppression of *P. australis* (e.g., those summarized by Kettenring & Adams, 2011; Lombard et al., 2012) could also be reduced with RPAS-based herbicide applications. The few surviving ramets observed in our study will mean that the area requiring follow-up treatment is reduced compared to helicopter application methods. Further, RPAS technology has valuable potential for follow-up spot spraying of remnant *P. australis* ramets after initial herbicide treatments. Minimizing repeated herbicide applications is crucial to reduce the total amount of herbicide entering wetlands for *P. australis* management and lowering risks of *P. australis* evolving herbicide resistance and the threat of herbicides accumulating in the environment.

2.5 Conclusions

RPAS-based herbicide application at Rondeau Provincial Park and Baie du Doré reduced live *P. australis* stem density by over 99% and canopy height by ~94% in treated plots. As expected, in the first year after herbicide application the diversity and evenness of the native plant community did not improve compared to control plots that were left untreated. The species richness, Shannon-Weiner diversity, Simpson's reciprocal diversity, and evenness, decreased (33%, 73%, 50%, and 73%, respectively) in the first year following treatment. While the mean coefficient of conservatism did not change after herbicide treatment, field-based observations suggested a shift away from *P. australis* dominance and towards the establishment of native species. Particularly in Baie du Doré, the native species *Impatiens capensis* came to dominate, however in Rondeau Provincial Park, the treated areas were dominated by *Hydrocharis morsus-ranae*. It is unclear what factors caused the different outcomes in the two wetlands, except that *H. morsus-ranae* was widespread in Rondeau Provincial Park but absent from the Baie du Doré plots in 2022 (before treatment), suggesting that pre-treatment surveys could help predict the risk of secondary invasions following *P. australis* suppression and supporting the call for long-term monitoring to assess the trajectory of native vegetation recovery in the years following the initial herbicide treatment (Kettenring & Adams, 2011; Hazelton et al., 2014). We conclude that RPAS-based herbicide application can control *P. australis* invasions in wetland environments with suppression efficacy as high or higher than any imazapyr-based *P. australis* suppression study reported in the literature (e.g., Mozdzer et al., 2008; Whyte et al., 2009; Knezevic et al., 2013). Future research should focus on evaluating the long-term effects of RPAS-based herbicide applications and monitoring the recovery of native plant communities over extended periods of time. Expanding the scope to larger patches of *P. australis* and varying environmental conditions will also be a critical step for refining management strategies. Addressing these knowledge gaps

will advance our understanding of the potential for RPAS technology in controlling invasive plants and contribute to more effective and sustainable wetland conservation efforts.

3.0 Effect of droplet size on coverage and drift potential of RPAS-based herbicide application on invasive *Phragmites australis*

3.1 Introduction

Wetlands serve as biodiversity hotspots (e.g., Ontario Ministry of Natural Resources and Forestry, 2017). Even with dense stands of invasive *Phragmites australis* ssp. *australis*, native vegetation often surrounds or grows interspersed within them. However, the indiscriminate nature of the broad-spectrum, systemic, herbicides used to suppress *P. australis* can cause unintended damage to non-target, native vegetation (Tu et al., 2001). Minimizing the collateral damage by herbicides used in invasive plant suppression is important because these native plants are critical sources of propagules to re-establish diverse native plant communities after treatment (Boutin et al., 2013). The primary goal of *P. australis* management when applying herbicides is to suppress all the *P. australis* to ensure minimal regrowth in the following season. A secondary goal is to minimize the extent of lateral drift or ground loss from the application to reduce non-target effects on neighboring native vegetation. These two goals can conflict, as fulsome suppression of *P. australis* can come at the cost of significant overtreatment, whereas restraint with herbicide application to minimize non-target effects can lead to undertreatment.

Both herbicides (imazapyr and glyphosate) approved for application to control invasive *P. australis* in Canada and the USA are systemic herbicides mixed with adjuvants that are absorbed by the plant's foliage and translocated into the stem and roots, damaging all plant tissues (Tu et al., 2001). To effectively suppress *P. australis* with herbicide, it is therefore critical to distribute the herbicide throughout the canopy. The dense (> 20 live stems /m²) and tall (> 2 m) growth form of *P. australis* (Chapter 2.0) may limit vertical penetration into the canopy and

foliage on shorter ramets may evade application entirely, compromising the overall suppression of the patch (Norris et al., 2002). Further, with aerial applications, greater deposition of herbicides at the top-most layer of a plant canopy can risk higher proportions of drift away from the targeted area (Webb, 2002). While lateral herbicide drift outside of the application area is a major concern in aerial applications (Butts et al., 2020), there are also concerns about the vertical penetration of herbicides through the *P. australis* canopy. Here too there is a trade-off, as the herbicide needs to fully penetrate the canopy to adequately treat each ramet, without too much product passing through the canopy as ground loss, where it can pollute the underlying soil or water and be wasted (Kudsk, 2002). Achieving the desired suppression levels (> 90%) during an initial treatment will minimize the amount of herbicide required in subsequent follow-up treatments, reducing the likelihood of herbicide resistance evolving and the risk of environmental accumulation when reapplications occur over the same area (Crowe et al., 2011). Yet, minimizing ground losses is also necessary, particularly with imazapyr, which has a half-life in soils of up to 268 days and the potential for some plant species to be sensitive to imazapyr residue for up to four years after initial application (Douglass et al., 2016). Any pesticide that passes through the target canopy into the soil or water beneath not only increases product loss, but also risks environmental contamination and toxicity to non-target organisms, such as native plants and algae (Breckels & Kilgour, 2018).

Remotely piloted aircraft systems (RPASs) represent an emerging technology for aerial spray applications (Dronova et al., 2021; Mohsan et al., 2023) that may provide effective *P. australis* suppression (Chapter 2.0) with reduced off-target impacts (Chapter 4.0). However, little is currently known about the ability of RPAS-based herbicide application to vertically penetrate the dense canopy of *P. australis*.

The propulsions generated from multiple RPAS rotors tend to contribute to significant downwash of herbicide (Chen et al., 2021; Zhan et al., 2022), leading to increased vertical movement of droplets into the canopy. While the downwash force enhances the penetration of herbicide droplets into the canopy, it can also increase the movement of stems, potentially promoting a more uniform distribution of the product across all layers of foliage. Downwash may be particularly useful in *P. australis* canopies where foliage is dense and shorter stems may be interspersed underneath the top of the canopy. However, when herbicides are pushed too far into the canopy valuable product is lost to the soil or water below the canopy. Wang et al. (2019) studied this phenomenon by comparing spray deposition of a backpack sprayer and a single-rotor RPAS sprayer with Mylar cards placed at the top and base of a wheat canopy. They aimed to quantify deposition along the upper canopy and product loss to the ground (ground losses). The study revealed that with the highest spray volume for both application methods, over 38% of the tracer was lost to the ground when sprayed with a backpack sprayer, while this value was only 5 – 12.6% for an RPAS sprayer (Wang et al., 2019). It is unknown, however, whether RPAS application methods will yield similarly improved performance in *P. australis* suppression, as *P. australis* is much taller than wheat (up to 5 m vs. < 1 m in canopy height) and has very rigid cane-like stems. More, depending on the configuration of nozzles (nozzles directly under rotor vs. along a boom), the downwash provided by the rotors may also contribute to lateral drift of herbicide (Biglia et al., 2022).

Herbicide droplet drift to areas outside the intended zone of application is another mechanism of product loss and potential non-target effects that aerial herbicide application methods risk. Drift is defined as the unintended movement of herbicide droplets away from the targeted area (Government of Canada, 2020), and is exacerbated in aerial herbicide applications

due to the increased flight heights (5 – 10 m above canopy) and speeds (> 15 m/s) necessary for the aircraft to fly over and treat the site (Frank et al., 1994; Yao et al., 2021). In Canada, helicopters are the only method permitted for aerial herbicide application to suppress *P. australis*, but the large swath widths from helicopters (25 – 30 m; Yao et al., 2021) make them best suited to treat large, homogenous patches of *P. australis* (Chapter 4.0). According to the Habitat® Aqua product label, product drift may be observed up to 450 m downwind of a helicopter application (BASF Canada Inc., n.d.).

Multirotor RPASs have enhanced maneuverability compared to helicopters, including the ability to turn in place and hover or fly at low heights close to the canopy (Martin et al., 2020), which may improve the ability of herbicide application to match the irregular contours of *P. australis* patches. They also have smaller swath widths than helicopters, reducing the extent of over herbicide applied to native plants growing around the *P. australis* patch (Martin et al., 2019). Droplet size is considered a key factor in droplet deposition of aerial spraying (Chen et al., 2020), among others including wind velocity, spray volume, and canopy structure. Both vertical canopy penetration and lateral drift potential concerns are sensitive to the droplet size used in aerial applications. Despite this, few have studied the effects of droplet size on pesticide deposition in actual field conditions. It has been shown that finer droplets increase spray coverage when spray volume is >15 L/ha (Shan et al., 2021), which may improve the deposition of product on long narrow surfaces (Ferguson et al., 2018), such as those of *P. australis* leaves. However, finer droplets, characterized by their small diameter (< 200 µm), are lighter and remain airborne for longer, leaving them exposed to lateral movement from the wind and increasing the chance of droplets being carried beyond the intended treatment area (Kruger et al., 2019). The drift potential of finer droplet sizes not only risks reduced suppression efficacy of the herbicide

application but also poses environmental concerns, including the potential for unintended exposure to non-target species. Interestingly, there is no universal regulation on droplet size for the application of systemic herbicides to *P. australis*. For example, the registration of an imazapyr-based herbicide from the United States Environmental Protection Agency (2010) states that coarse (341 – 403 μm ; American Society of Agricultural and Biological Engineers, n.d.) or very coarse (404 – 502 μm ; American Society of Agricultural and Biological Engineers, n.d.) droplet sizes are required for aerial herbicide applications that are released below 10 feet (3 m), despite improved coverage from finer droplets (Shan et al., 2021). Drift management on the Habitat® Aqua label, however, indicates that the product should, at the smallest, be a medium droplet size (236 – 340 μm ; American Society of Agricultural and Biological Engineers, n.d.; BASF Canada Inc., n.d.). Therefore, assessing the droplet deposition of these droplet sizes is critical prior to implementing RPAS technology in wetland environments to ensure adequate spray coverage can be achieved throughout the *P. australis* canopy, and to determine the extent of drift in order to minimize damage to non-target vegetation.

We examined the effect of droplet size on herbicide droplet deposition when applied by an RPAS to a *P. australis* canopy and compared the drift potential away from the application area. Our objectives were: 1) to evaluate vertical penetration of herbicide through the plant canopy, characterized by percent area coverage and volume median diameter, at three different heights (approximately 1.5 m, 2 m, and 2.5 m) within a *P. australis* canopy and 2) to assess the extent of lateral spread via droplet drift outside (0 – 8 m) the target application area. Since droplet size is expected to influence vertical penetration and lateral drift potential, we compared medium (236 – 340 μm ; American Society of Agricultural and Biological Engineers, n.d.; BASF

Canada Inc., n.d.) and coarse (341 – 403 μm ; American Society of Agricultural and Biological Engineers, n.d.) droplet sizes.

3.2 Methods

3.2.1 Study design

To advance these objectives, we employed an in-field herbicide application experiment, manipulating droplet size to assess vertical canopy penetration and lateral droplet drift of RPAS-based herbicide application on a homogenous patch of *P. australis* when applying medium (236 – 340 μm ; American Society of Agricultural and Biological Engineers, n.d.) or coarse (341 – 403 μm ; American Society of Agricultural and Biological Engineers, n.d.) droplet sizes. We used water-sensitive papers (SpotOn, Innoquest inc., Woodstock, IL, USA) as indicators to capture area coverage and volume median diameter of the aqueous herbicide droplets during application, within and around the *P. australis* patch.

3.2.2 Field site

Our study site is situated within the Pinery Provincial Park in Grand Bend, Ontario is located within the municipality of Lambton Shores. Pinery Provincial Park provides critical habitat for several provincially and nationally recognized species at risk (Flowers et al., 2023). Our experiment focused on an isolated patch of invasive *P. australis* (~1.3 ha) located near the southernmost end of the Old Ausable Channel, which traverses the park (Fig. 3-1). This southern portion of the channel comprises a Provincially Significant Coastal Wetland: a swamp-marsh complex (Port Franks Wetland (AB-12), evaluated ID: 651 138 092). The study area is surrounded primarily by shallow open water, with few species of emergent and floating

vegetation present (e.g., *Pontederia cordata*, *Lemna minor*, and *Nymphaea odorata*). The channel encompasses the *P. australis* patch on the north, east, and south sides, inhibiting access for backpack sprayers, while the western side of the patch is bounded by Carolinian forest, hindering application by helicopters or planes due to the risk of overspray affecting the trees.

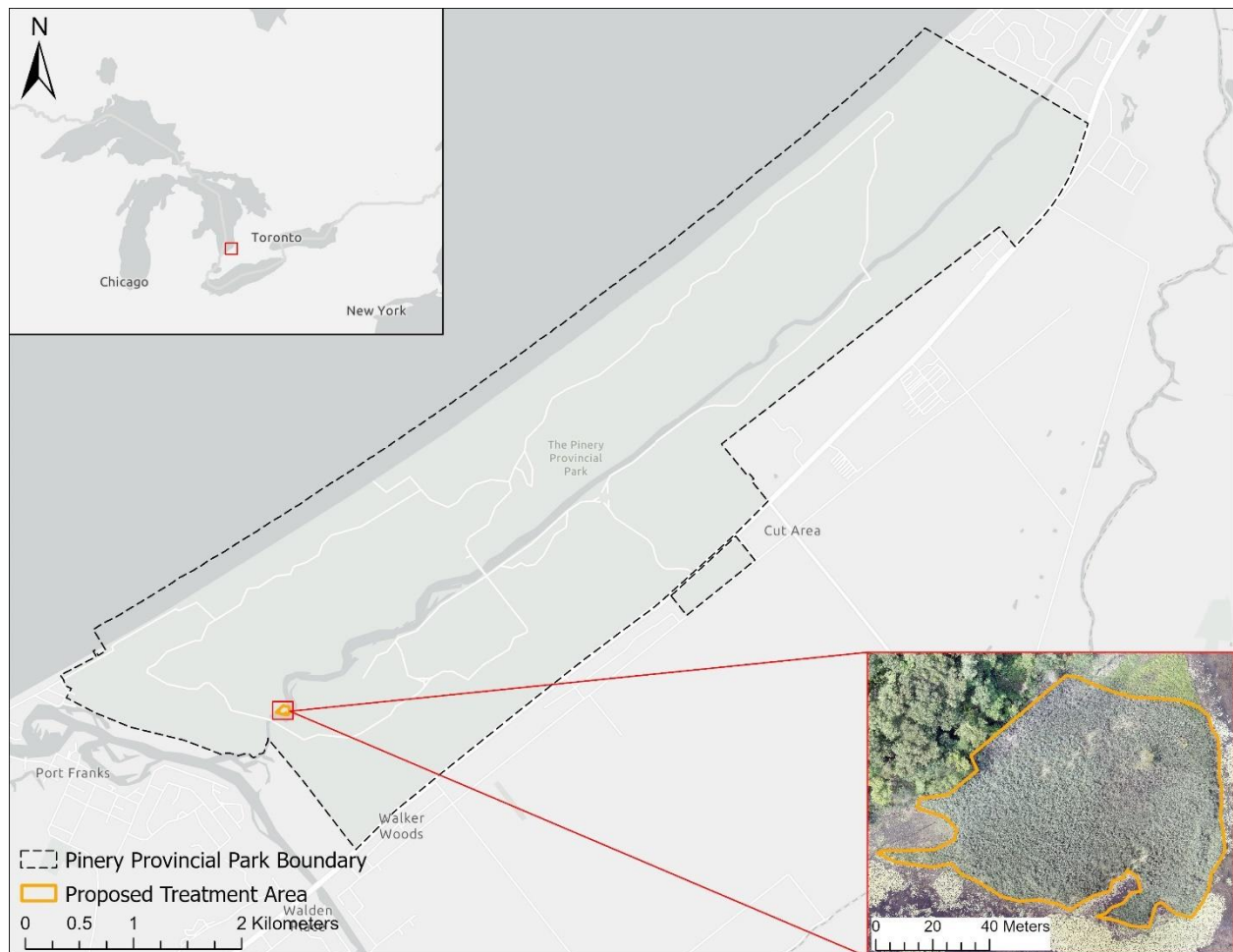


Figure 3-1. Map of *Phragmites australis* patch proposed for Habitat® Aqua application by an RPAS, in relation to the Pinery Provincial Park boundaries. Basemap imagery of the inset map was collected by Ontario Power Generation’s Advanced Inspection and Maintenance team in 2023.

3.2.3 RPAS-based herbicide application

In October 2023, imazapyr as an isopropylamine salt (concentration 240 g/L), under the tradename Habitat® Aqua (BASF Canada Inc., Pesticide Registration Number 32374; Proposed

Registration Decision PRD2020-17), was applied to the treatment area by an RPAS under the Research Authorization 0009-RA-22. The herbicide was applied at a rate of 4.68 L/ha and total spray volume of 100 L/ha (includes 0.25% v/v Aquasurf® non-ionic spray adjuvant; Norac Concepts Inc., Pesticide Registration Number 32152), by a licensed drone pilot and pesticide applicator (L-208-8129866762). The RPAS used for application was a quadcopter (DJI, Guangdong, China), equipped with four TT110015 nozzles (one per rotor). Pressure was manipulated so that one half of the patch was sprayed with a medium droplet size (236 – 340 µm; American Society of Agricultural and Biological Engineers, n.d.) using a pressure of approximately 40 PSI and the other half was sprayed with a coarse droplet size (341 – 403 µm; American Society of Agricultural and Biological Engineers, n.d.) using a pressure of approximately 20 PSI. When pressure is decreased, droplet size distribution will shift towards a larger size, allowing us to switch between medium and coarse droplets (Kooij et al., 2018). The RPAS flew at a height of ~3 m above the *P. australis* canopy and speed of 1.7 m/s for both droplet size missions.

Application procedures adhered to the Habitat® Aqua label recommendations for reducing spray drift and weed-specific instructions for *P. australis* (BASF Canada Inc., n.d.). In the field, treatment areas were delineated by flying the RPAS over the patch with the pilot visually examining the perimeter through the first person-view camera. A dividing border perpendicular to the tree line was established to create two treatment areas covering the patch, with one treatment area being randomly designated for each droplet size (Fig. 3-2). Visual observations in the field showed no evidence of spray drift due to wind gusts as the rotors of the RPAS primarily pushed the herbicide droplets downwards. We recorded weather parameters at the site before and during each droplet size mission; temperature and relative humidity were

measured with a handheld hygro-thermometer (Extech RH300, Extech Instruments, Waltham, MA, USA) and ranged between 25.9 – 27.6 °C and 48.8 – 55.3%, respectively. Wind direction, wind velocity, and wind gusts were measured with a handheld anemometer from ~1 m off the ground (Kestrel 1000 wind meter, Kestrel Instruments, Boothwyn, PA, USA) and were south-southwest, 1.1 – 2.2 kph, and 11.2 – 13.0 kph, respectively.

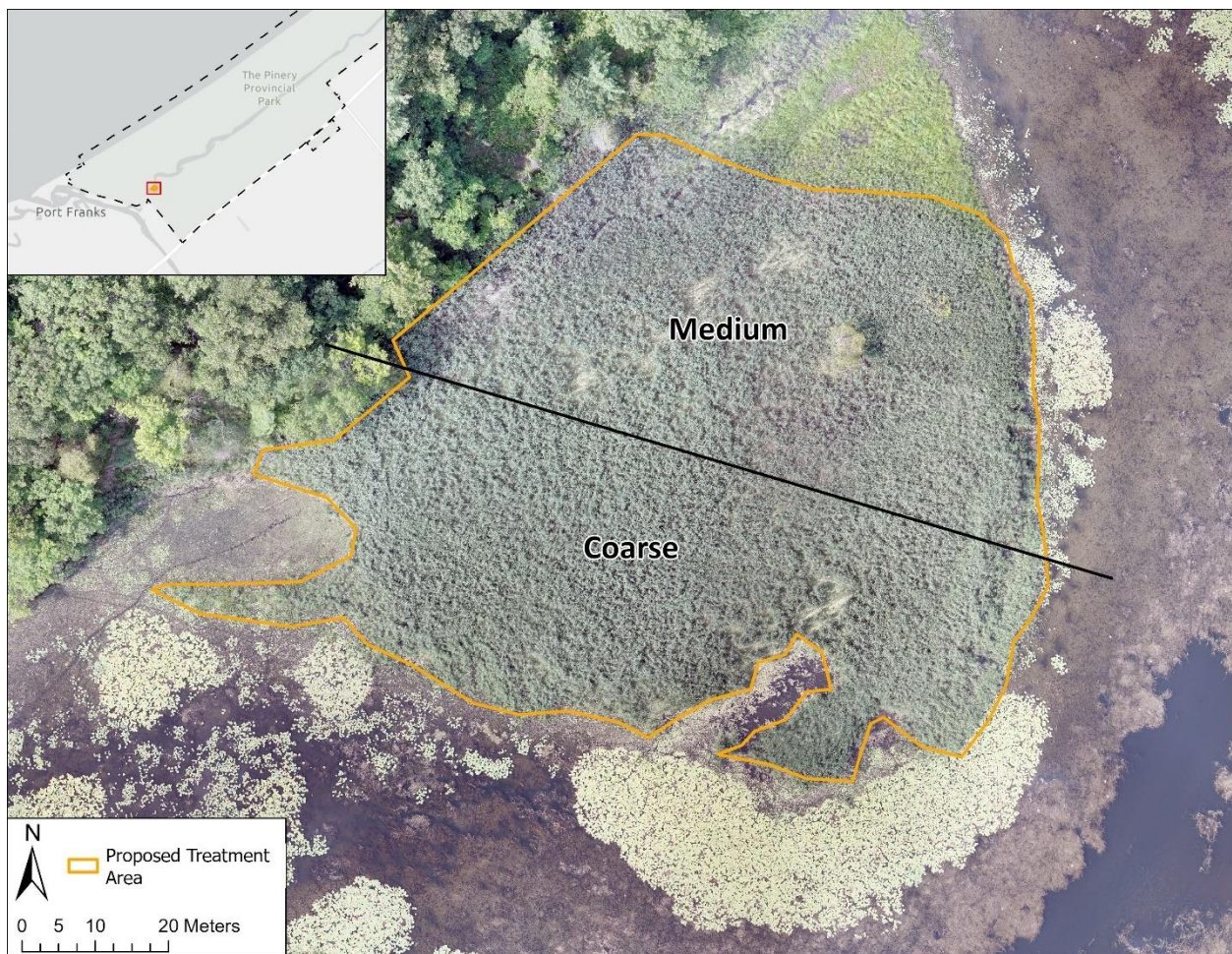


Figure 3-2. Map of proposed treatment area split into two treatment areas. The top half of the proposed treatment area was designated for medium droplet application (236 – 340 μm ; American Society of Agricultural and Biological Engineers, n.d.) by the RPAS, and the bottom half of the proposed treatment area was designated for coarse droplet application (341 – 403 μm ; American Society of Agricultural and Biological Engineers, n.d.). The black line represents the dividing border between the two droplet sizes. Basemap imagery was collected by Ontario Power Generation’s Advanced Inspection and Maintenance team in 2023.

3.2.4 Field methods

Field preparation

We pre-labelled water-sensitive papers with a code corresponding to treatment type (medium or coarse droplet size) and location in or around the *P. australis* patch (top, middle, or lower leaves on a *P. australis* ramet or incremental distance away from patch edge). We also placed small marker stickers on the back of each paper to label their treatment type and location, in case moisture from the aqueous herbicide or humidity in the field caused bleeding of the written labels. We grouped the water-sensitive papers into labelled Ziploc bags by treatment type and location to facilitate deployment of papers in the field. We followed recommendations from Hypro (2006) including wearing Nitrile gloves and handling water-sensitive papers by the edges to minimize potential contamination, both during the labelling process as well as during the field set-up and take-down of papers.

Field set-up

Canopy penetration

We selected a total of six areas (half, $n=3$ for each droplet size) located sufficiently inside the *P. australis* patch (> 3 m from edge) to ensure penetration would be measured from the interior, without effects from the ramets along the edge of the patch (Fig. 3-3). Within each area, we selected five ramets within 1 m^2 and marked them with flagging tape to be easily identified after the herbicide application. To capture the range of herbicide droplets within the *P. australis* canopy, we stapled 1"x 3" water-sensitive papers (SpotOn, Innoquest inc., Woodstock, IL, USA) to the center of *P. australis* leaves, ensuring that each paper was located entirely inside the bounds of the leaf. We secured a paper to a lower (~ 1.5 m), middle (~ 2 m), and top (~ 2.5 m) *P.*

australis leaf on each of the ramets, measuring the height of each paper above the water to the nearest centimeter once it was secured.

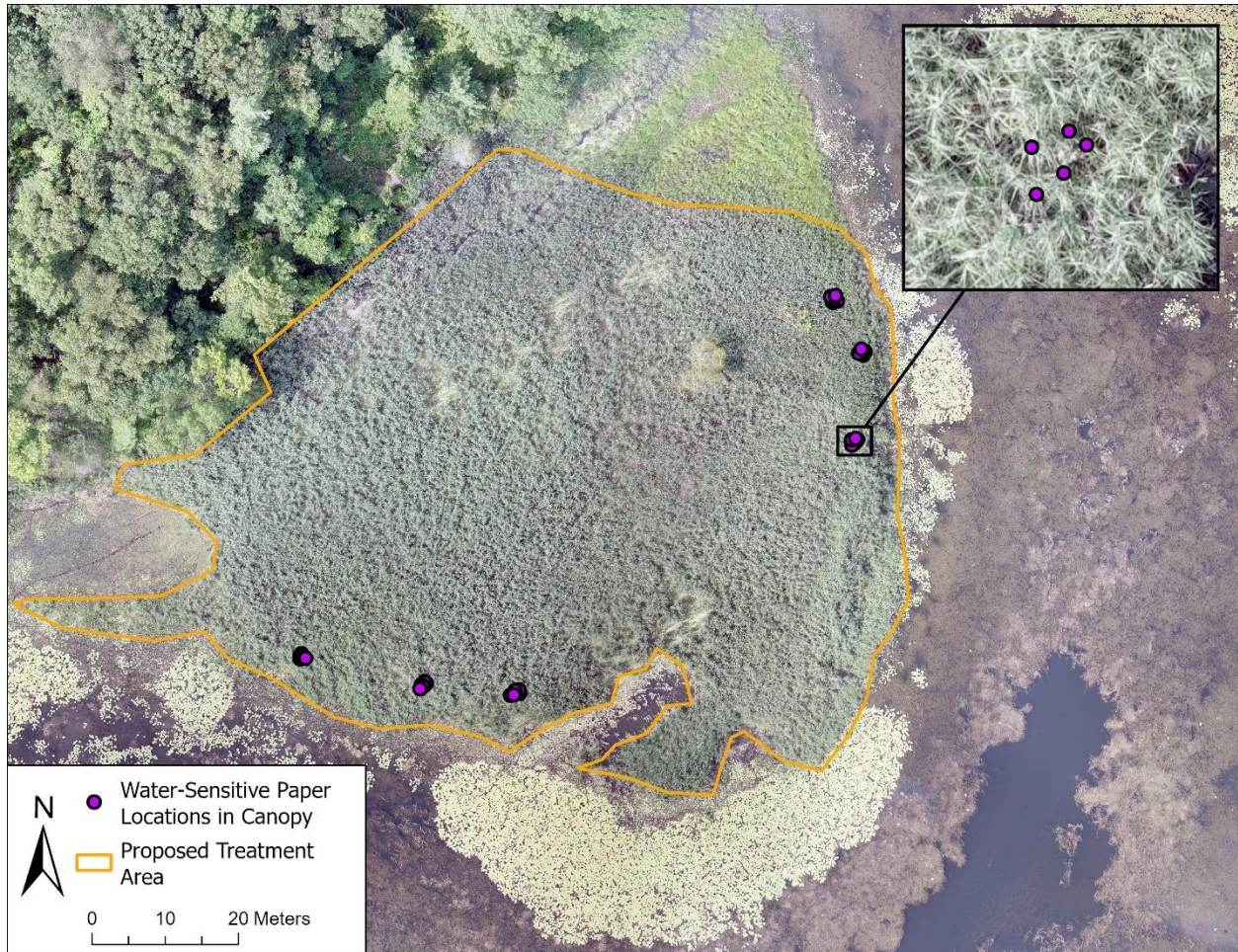


Figure 3-3. Locations of water-sensitive papers in *Phragmites australis* canopy. Three areas were established for each droplet size, with water-sensitive papers being attached to five ramets in each area (see inset). Water-sensitive papers for the medium droplet size (n=45) and coarse droplet size (n=45) were secured to a lower (~1.5 m), middle (~2 m), and top (~2.5 m) *P. australis* leaf. Basemap imagery was collected by Ontario Power Generation’s Advanced Inspection and Maintenance team in 2023.

Drift

To capture evidence of drift, we established a total of ten transects around the patch with half (n=5) along the boundary of the patch that was designated to be sprayed with medium droplets and half along the boundary of the patch that was designated to be sprayed with coarse

droplets (Fig. 3-4). At incremental distances along each transect (2 m, 4 m, 6 m, and 8 m), radially from the edge of the *P. australis* patch, we placed 2"x 3" water-sensitive papers (SpotOn, Innoquest inc., Woodstock, IL, USA) ~10 cm above the water level, using U-channel posts. We secured a wire card holder to the top of each U-channel post and then into each card holder we placed a 2"x 3" water-sensitive paper with the indicator surface oriented upwards and slightly toward the *P. australis* patch margin, as close to the horizontal plane as the wire card holders could be bent (Fig. 3-5). Median values for our response variables were taken from the water-sensitive papers used for the canopy penetration experiment, from the top height to substitute a value for 0 m distance from the application area. This approach eliminated any effects of scattering or droplet interception from the ramets along the edge of the *P. australis* patch that could yield artifacts in the response variables if papers were staked right at the patch edge.

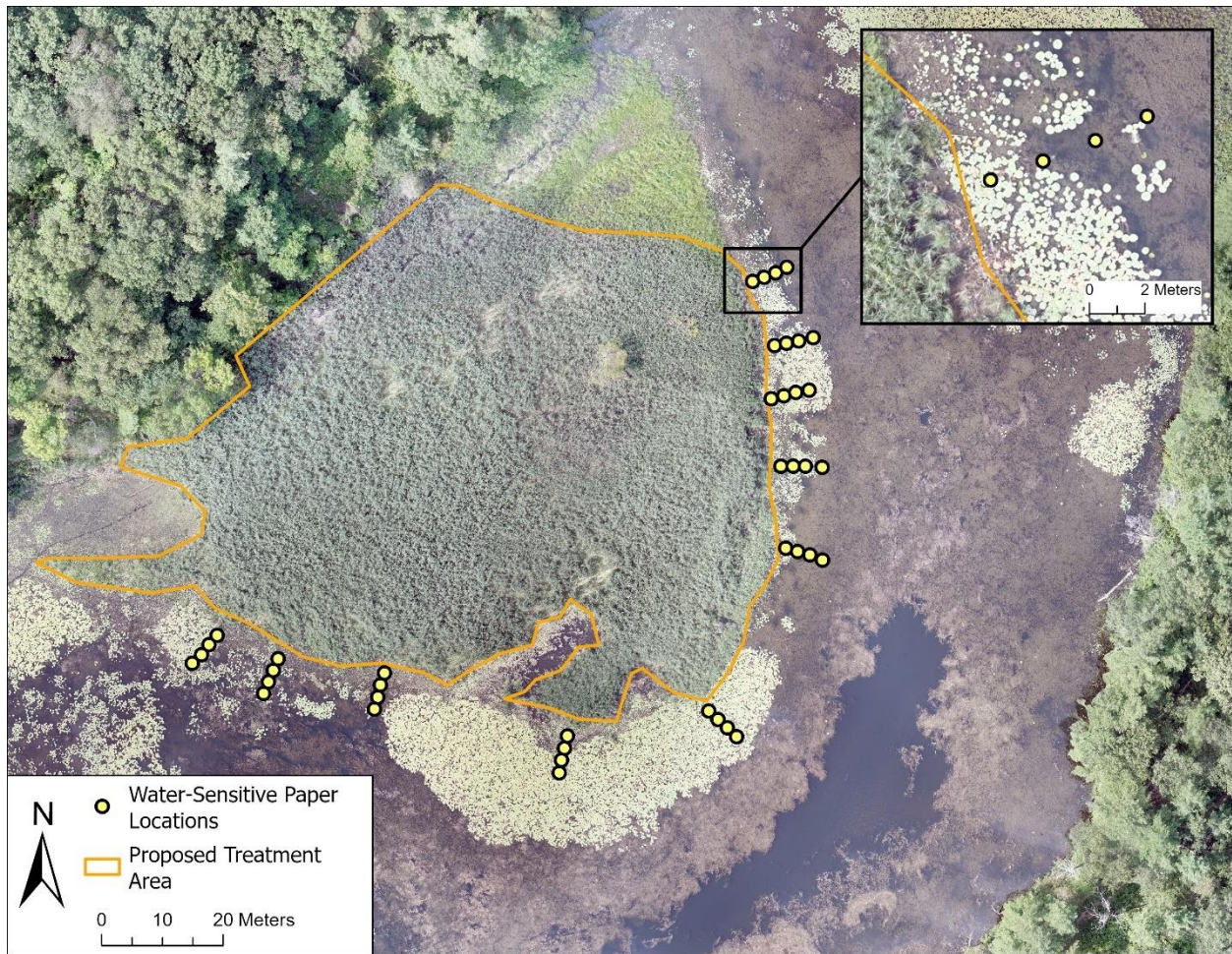


Figure 3-4. Locations of water-sensitive papers around *Phragmites australis* canopy. Five transects were established for each droplet size, with water-sensitive papers secured at 2 m intervals away from the proposed treatment area (see inset). Water-sensitive papers for the medium droplet size (n=20) and coarse droplet size (n=20) were secured at 2 m, 4 m, 6 m, and 8 m away from the patch. Basemap imagery was collected by Ontario Power Generation's Advanced Inspection and Maintenance team in 2023.

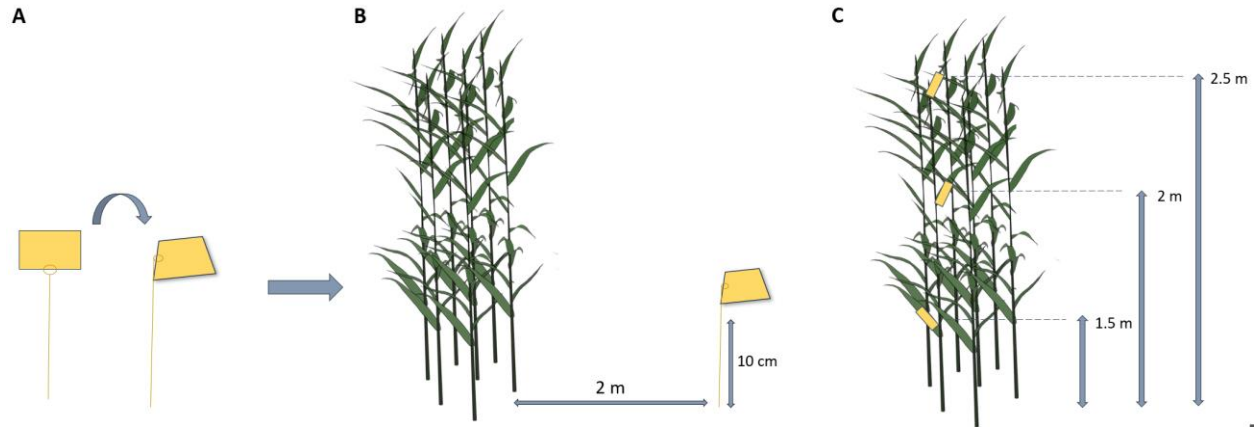


Figure 3-5. Graphic of water-sensitive paper, placed in wire card holder and bent towards the horizontal plane (A), water-sensitive paper placement in proximity to the *Phragmites australis* patch (B), and water-sensitive paper place within the *P. australis* patch (C).

Field take-down

We followed precautions on the Habitat® Aqua product label and waited for the herbicide spray to be completely dry before collecting water-sensitive paper in the field (BASF Canada Inc., n.d.). We collected the water-sensitive papers from the wire card holders along a transect and imaged the papers from a fixed distance of 15 cm in the field in case of contamination or accidents during transportation to the lab. After imaging the papers from a transect, we placed them into their labelled Ziploc bag, making sure that none of the indicator surfaces were in contact with each other. We then returned to all six areas in the *P. australis* canopy where papers were secured and carefully removed all water-sensitive papers from the leaves. We collected water-sensitive papers from each ramet and placed them back into their respective labelled Ziploc bag. Due to time constraints in the field, water-sensitive papers within the *P. australis* canopy were only imaged in the lab.

3.2.5 Data extraction

One day after the herbicide treatment, we scanned the water-sensitive papers with a Lexmark MC3326 Laser multifunction printer (Lexington, KY, USA) at an image resolution of 600-dpi. We extracted values of area coverage (%) and volume median diameter (VMD; μm) of the herbicide droplets on each paper using DepositScan software (USDA, Wooster, OH, USA; Zhu et al., 2011). Due to the two-dimensional nature of water-sensitive papers, any overlapping droplets would be interpreted as a single large droplet, rather than multiple overlapping individual droplets, underrepresenting the true value of droplet density (Salyani et al., 2013). In a comparison of several water-sensitive paper analysis systems (DepositScan, Conta-Gotas, CIR, and e-Sprinkle), Cunha et al. (2013) found that area coverage was the most consistently reported measurement, while droplet density was the least consistent. Values of droplet density were not reported in our analyses as the values could not be accurately characterized or compared with existing literature.

3.2.6 Analytical methods

To first test if the coverage and VMD of herbicide droplets were significantly different ($\alpha = 0.05$) throughout the *P. australis* canopy, we conducted a Kruskal-Wallis test (Kruskal & Wallis, 1952) with each droplet size (medium and coarse) using height within the canopy (categorized as lower, middle, and top) as the predictor variable against each response variable (area coverage and VMD). We used the nonparametric equivalent to a one-way ANOVA, as our data violated the assumptions of the parametric test. To test if droplet size had a significant effect ($\alpha = 0.05$) on the area coverage and VMD of herbicide droplets at various heights within *P. australis* canopy, we used Wilcoxon signed-rank tests (Wilcoxon, 1945). Paired observations

were selected within each height category (lower, middle, or top) to control for variability in canopy height. We used the nonparametric equivalent of a paired t-test as the median values of our response variables better represented the center of the data distribution, compared to the mean, and to maintain a consistent comparison among results of the Kruskal-Wallis test. All statistical analyses were conducted in R v. 4.3.2 (R Core Team, 2023).

To determine the spatial extent of drift beyond the application area, we quantified the relationship between our response variables and distance from the application area. We examined the effect of droplet size on our response variables at incremental distances from the application area by calculating the median value of each response variable (area coverage and VMD) at each distance from the *P. australis* patch (0 m, 2 m, 4 m, 6 m, 8 m). We fit an exponential decay curve (i.e., $y = y_0 e^{-k \cdot x}$) to the median values of our response variables for each droplet size and computed corresponding decay constants ($\alpha = 0.05$) for each curve. All analyses were conducted in R v. 4.3.2 (R Core Team, 2023).

3.3 Results

Results suggest that canopy penetration of the RPAS-based herbicide application was relatively consistent, regardless of which droplet size was used. We assessed canopy penetration on dense (~60 live stems/m²), homogenous, *P. australis* through response variables including percent area coverage and volume median diameter (VMD) of herbicide droplets on water-sensitive papers fixated at different leaf heights on *P. australis* ramets. Percent area coverage (Fig. 3-6A) tended to be right-skewed, with a few samples having much higher coverage with herbicide than the bulk of samples. This variable did not differ among lower, middle, and top leaf heights when the RPAS sprayed with medium (Kruskal-Wallis: $H_2 = 0.155$, $p = 0.925$) or

coarse droplets (Kruskal-Wallis: $H_2 = 1.27$, $p = 0.530$). The VMD (Fig. 3-6B) also did not have a significant difference among lower, middle, and top leaf heights with medium (Kruskal-Wallis: $H_2 = 0.290$, $p = 0.865$) or coarse droplets (Kruskal-Wallis: $H_2 = 0.239$, $p = 0.887$).

Overall, there were few differences between the medium and coarse droplet sizes, in terms of percent area coverage or VMD. Differences in our response variables were assessed between droplet sizes (medium and coarse) at lower, middle, and top leaf heights on *P. australis* ramets (Fig. 3-6). There was no significant difference in percent area coverage between coarse and medium droplet sizes at the lower (Wilcoxon: $W = 80$, $p = 0.277$), middle (Wilcoxon: $W = 56$, $p = 0.847$), or top (Wilcoxon: $W = 57$, $p = 0.808$) leaf heights in the *P. australis* canopy. Volume median diameter between coarse and medium droplets was not significantly different at middle (Wilcoxon: $W = 77$, $p = 0.359$) or top (Wilcoxon: $W = 66$, $p = 0.426$) leaf heights. However, the VMD of medium droplets was found to be significantly smaller at the lower leaf height than coarse droplets (Wilcoxon: $W = 99$, $p = 0.026$).

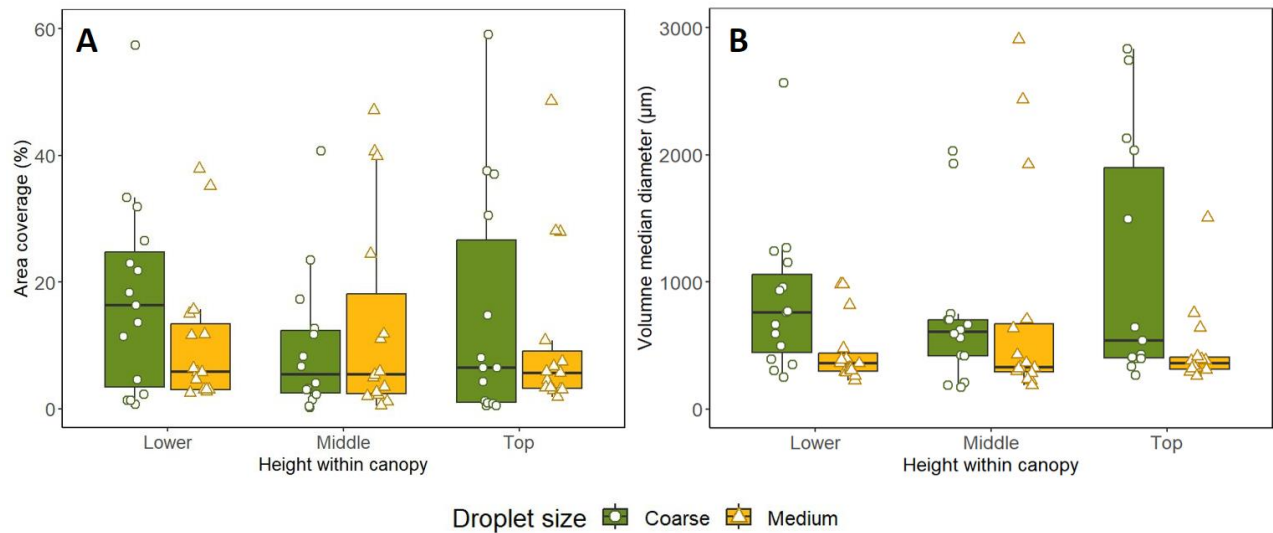


Figure 3-6. Boxplot comparison of percent area coverage (A) and volume median diameter (B) at different heights within a *Phragmites australis* canopy from an RPAS-based herbicide application using coarse and medium droplet sizes. Lower, middle, and top heights within canopy represent, on average, 1.5, 2, and 2.5 meters above water level, respectively. Green boxes and circles represent water-sensitive papers sprayed with coarse droplets, and yellow boxes and triangles represent water-sensitive papers sprayed with medium droplets. Created with ggplot2 (Wickham, 2016).

We assessed herbicide drift from the RPAS-based application using medium and coarse droplet sizes on *P. australis* through response variables of percent area coverage and VMD of herbicide droplets on water-sensitive papers fixated at two-meter intervals away from the application area (Fig. 3-7). The median value of percent area coverage was approximately 6% within the application area (i.e., within the interior of the patch of *P. australis*) for medium and coarse droplet sizes. Both droplet sizes displayed an exponential decrease in percent area coverage with distance from the *P. australis* patch; decay constants for the medium and coarse droplet sizes were 0.397 ($p < 0.001$) and 0.211 ($p = 0.019$), respectively. Corresponding decay rates are 0.328 for medium droplets and 0.190 for coarse droplets, representing approximately a 33% and 19% decrease in percent area coverage, respectively, per meter distance away from the application area.

Median values of VMD for medium and coarse droplets had a dramatic exponential decrease with distance away from the patch. The decay constant for the VMD of medium droplets was 0.177 ($p = 0.033$) with a corresponding decay rate of 0.162, representing a decrease of approximately 16% per meter distance away from the application area. While the decrease in VMD for coarse droplets was slightly greater in magnitude with a decay constant of 0.237 ($p = 0.015$) and a corresponding decay rate of 0.211, representing a decrease of approximately 21% per meter distance away from the application area. Table 3-1 provides a summary of the decay coefficients extracted from the exponential decay curves for percent area coverage and VMD.

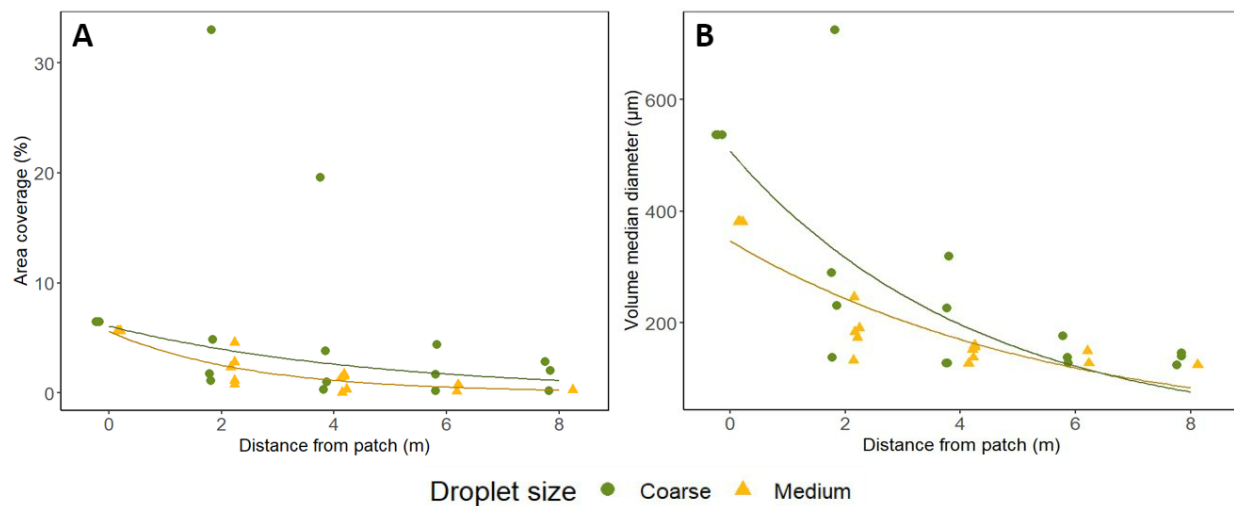


Figure 3-7. Exponential decay curves fitted to median values of percent area coverage (A) and volume median diameter (B) at increasing distances away from a *Phragmites australis* patch from an RPAS-based herbicide application using coarse and medium droplet sizes. Green circles represent coarse droplets, yellow triangles represent medium droplets. Created with ggplot2 (Wickham, 2016).

Table 3-1. Summary of decay coefficients extracted from exponential decay curves for percent area coverage and volume median diameter. Coefficients fit the equation $y = y_0 e^{-k \cdot x}$ where y is the response variable, y_0 is the initial value of a given response variable, k is the decay constant, and x is the distance from the *Phragmites australis* patch in meters. Decay rate was calculated as $1 - e^{-k}$, where k is the decay constant.

Response variable	Droplet size	y_0	p value	k	p value	Decay rate
Percent area coverage	Medium	5.589	<0.001	0.397	<0.001	0.328
	Coarse	6.085	0.003	0.211	0.019	0.190
Volume median diameter	Medium	346.935	0.004	0.177	0.033	0.162
	Coarse	508.037	0.002	0.237	0.015	0.211

3.4 Discussion

Our study explored herbicide droplet deposition when applied by an RPAS to a dense *P. australis* canopy. We aimed to evaluate vertical penetration of herbicide through a *P. australis* canopy, at three different heights (lower, middle, and top), and to assess the extent of lateral spread via droplet drift up to 8 m beyond the target application area. We also examined the role of droplet size on vertical penetration and lateral drift potential, by comparing medium and coarse droplet sizes. Herbicide droplet deposition in terms of percent area coverage was not different among heights within the *P. australis* canopy for either droplet size, nor did droplet size have a significant effect on the vertical penetration. The exception to this was that the VMD of the coarse droplets was larger than the medium droplets at the lowest canopy height. This was in line with previous observations of vertical penetration of coarse droplets on a wheat canopy (Wang et al., 2019), mainly driven by the combined gravitational and rotor propulsion forces acting on the larger droplets. Overall, rotor propulsion contributed to even dispersion of herbicide droplets through the canopy. Anecdotally, we attribute this to downwash-driven movement of the *P. australis* foliage during application. Lateral droplet drift occurred at least 8 m away from the application area, even under relatively low environmental wind speeds.

Unexpectedly, outside of the application area, medium droplets had a greater decay rate for percent area coverage and VMD compared to coarse droplets. These findings contradict the idea that smaller droplets have higher drift potential due to their lower mass and higher susceptibility to wind displacement. Although, the drift observations in this study suggest that RPAS downwash forces might have a greater influence on the displacement of herbicide droplets compared to environmental wind, especially when wind speeds are low (1.1 – 2.2 kph). This is because at low wind speeds, the downwash may more effectively push smaller droplets downward, towards the canopy, before wind is able to displace the droplets.

Canopy penetration observed in this study indicates that RPAS-based herbicide application can effectively reach target foliage through the canopy, even within dense stands of *P. australis*. Our results suggest that droplet size had limited influence on spray coverage across the vertical profile of the *P. australis* canopy. Both medium and coarse droplet sizes exhibited similar percent area coverage values at all three heights within the canopy, suggesting the choice of droplet size may have minimal impact on penetration through the canopy and the resulting suppression efficacy from an RPAS-based herbicide application. Similar results were reported by Chen et al. (2020) who compared rice canopy penetration of very fine (95.21 μm), fine (121.43 – 147.28 μm), and medium (185.09 μm) droplets using an RPAS and observed no significant differences in droplet penetration rate among the droplet sizes. A ground-based pesticide application study conducted by Ferguson et al. (2016) on oat canopy penetration examined the coverage across nearly the entire range of droplet sizes available for use in spray applications (very fine: 60 – 145 μm to ultra coarse: > 665 μm ; American Society of Agricultural and Biological Engineers, n.d.). They concluded that the trend between droplet size and percent area coverage at the ground, lower, middle, and top heights within the canopy was unclear (Ferguson

et al., 2016). While their study reported differences in spray coverage between the most extreme droplet sizes (very fine compared to ultra coarse) throughout the canopy, the practical range for post-emergent herbicide use is much smaller, with the recommended droplet size for most herbicides being coarse (American Society of Agricultural and Biological Engineers, n.d.), and for Habitat® Aqua specifically being medium (BASF Canada Inc., n.d.). Therefore, it is understandable that differences between medium and coarse droplet sizes in terms of percent area coverage throughout the *P. australis* canopy were not detected in our study.

The volume median diameter (VMD) results were similar to the percent area coverage results in terms of there being little difference in canopy penetration between the two droplet sizes. The exception is that at the low canopy height, the VMD was significantly greater for the coarse droplet size than the medium droplet size. The literature suggests this could be because coarse droplets experience greater downward momentum than medium sized droplets due to the combined gravitational and downward propulsion forces from the rotors (Dengeru et al., 2023). The combination of forces might be responsible for the larger sized droplets providing greater VMD at the lowest canopy height. Additionally, the greater liquid volume of larger droplets makes them less susceptible to evaporation from the time they are discharged from the RPAS to when they reach the targeted foliage (Wolf, 2000), increasing the time for a given droplet to penetrate the lower canopy layers. This indicates that while coarse droplets provided adequate vertical penetration throughout all canopy layers, the larger sized droplets at the lowest height in the canopy might lead to more ground losses if greater proportions of the herbicide are passing through the canopy without intercepting foliage.

In the field, we remarked that rotor propulsions not only produce a downward force for the herbicide droplets but also contribute to significant foliage movement in the upper canopy.

This additional movement during herbicide application may have contributed to herbicide droplets being deposited equally at the middle and lower heights, despite the dense canopy of *P. australis*. Our observed herbicide droplet deposition contrasts that of ground-based applications, where most of the herbicide is deposited on the top layer of the canopy (Ferguson et al., 2016). While the downwash forces and foliage movement can contribute to even herbicide deposition throughout the *P. australis* canopy, variation in rotor propulsion throughout the mission could impact the overall distribution of herbicides (Zhan et al., 2022). When full, a 10 L tank holding the herbicide can add an extra 10 kg of weight to the RPAS's payload. As the tank empties during herbicide application, the payload decreases steadily. Since the RPAS is programmed to maintain a consistent flight height and speed during the mission, propulsion from the rotors needs to remain dynamic to account for the changing weight of the payload (Zhan et al., 2022). The shifts in propulsion necessary to maintain flight height and speed will lead to changes in downwash forces such that the proportion of herbicide pushed into the canopy will be greatest when propulsion is high and the tank is full, versus when propulsion is reduced by the herbicide tank emptying (Zhan et al., 2022). Flight plans should account for this variation by considering where the influence of downwash on droplet deposition and drift potential will be most valuable over the *P. australis* patch.

Consistent droplet deposition across the range of heights examined (1.5 – 2.5 m above water level) supports RPAS technology as a suitable herbicide application method for dense *P. australis* canopies. Combined with the results from Chapter 2.0, this suggests that coverage throughout the canopy is sufficient for effective suppression to be achieved. There was no systematic difference in percent area coverage or VMD with canopy height, regardless of which

droplet size was used, as these variables yielded remarkably consistent measures among the three canopy heights assessed.

While adequate leaf coverage throughout the plant canopy is important to ensure treatment efficacy and *P. australis* suppression, too much force could push droplets through the canopy and into the water or sediment below. When herbicide droplets pass through the plant canopy, it is termed ground loss because product is lost to the ground and does not intercept foliage, to suppress the target plant. We did not see any trends in VMD or percent area coverage with depth through the canopy, suggesting some product likely passed through the *P. australis* canopy in our study. Wang et al. (2019) reported that RPAS-based pesticide spraying had significantly less product ground loss when compared to backpack spraying on a wheat canopy. Although, they did find that deposition from the RPAS treatment increased at ground-level when spraying with coarse droplets compared to medium droplets. While we did not directly assess ground loss in our study, the difference in VMD between droplet sizes at the lower canopy height supports the Wang et al. (2019) results. To further refine the flight parameters for RPAS-based herbicide application to *P. australis* in wetlands, we recommend quantification of ground losses, as significant ground loss can lead to unwanted herbicide accumulation (Douglass et al., 2016), increased non-target vegetation damage (Breckels & Kilgour, 2018), and economic loss.

Across nearly all heights within the *P. australis* canopy, we observed that the medium droplet size performed more consistently than the coarse droplet size, which exhibited greater variability in area coverage and VMD. This was especially evident at the top-most height within the canopy, where coarse droplets exhibited considerably greater variance in both percent area coverage and VMD. These observations contrast those of Chen et al. (2020), who reported medium droplet sizes as having lower coefficients of variance compared to fine or very fine

droplets, suggesting that uniformity should increase with droplet size. Decreasing the size of the droplets directly increases the number of droplets produced at the same spray volume (Hofman & Solseng, 2004), making smaller droplets favorable due to the even coverage afforded by their size. Further, a greater number of droplets sprayed from the RPAS increases the chance of successful interception by the target foliage, which may be more valuable in RPAS applications where the rotor propulsions move the canopy around. When droplet sizes are larger, interception of the targeted foliage becomes more challenging to predict as the greater mass increases the momentum of the droplet and may lead to the droplet bouncing or running off the foliage (Ferguson et al., 2018). Additionally, having larger but fewer droplets means that the interception of each droplet becomes more influential in maximizing suppression efficacy of the treatment. Additional studies on droplet size variation under field conditions should be completed to determine what factors influence performance consistency with RPAS-based herbicide applications.

Contrary to our expectations, medium droplets demonstrated a greater decay rate for percent area coverage and VMD compared to coarse droplets. Our results suggest that medium droplets had less drift potential than coarse droplets when applied by an RPAS to a *P. australis* canopy. For a given distance away from the application area, fewer medium droplets tended to drift compared to the coarse herbicide droplets, contradicting the widely reported relationship between droplet size and droplet drift in the literature (e.g., Hunter et al., 2019; Gibbs et al., 2021; Grant et al., 2022). For example, Chen et al. (2020) assessed the drift rate of very fine to medium droplets sprayed from an eight-rotor RPAS and reported a distinct decrease in drift rate with increased droplet size. As droplet size decreases, surface area to volume ratio increases (Hofman & Solseng, 2004), and the influence of air resistance becomes greater, increasing drift

opportunity. Smaller droplets are lighter and are expected to remain suspended in the air column for longer, increasing the chance for wind displacement (Kruger et al., 2019). However, high drift potential is most likely to occur when droplet sizes are $< 200 \mu\text{m}$ (Hofman & Solseng, 2004). Therefore, it is possible that differences in drift potential were more challenging to capture between the medium and coarse droplets, where 50% of the droplets are greater than $236 \mu\text{m}$ and $341 \mu\text{m}$ (American Society of Agricultural and Biological Engineers, n.d.), respectively. The medium droplets are more susceptible to evaporation while airborne (Kruger et al., 2019), potentially lowering the chances of droplets landing on the indicators or having enough liquid present to stain the papers. One potential explanation for our unexpected finding is that droplet size was achieved through different pressure rather than different nozzle ends. The medium droplets were created by pushing 40 psi through the lines, whereas the coarse droplets were created by reducing the pressure to 20 psi. This higher pressure may have helped propel the medium droplets downward, reducing the opportunity for drift. A limitation to our water-sensitive papers method that bears noting is that droplets less than $50 \mu\text{m}$ can be too small to create a measurable stain on water-sensitive papers (Hoffmann & Hewitt, 2005). It follows that the proportion of detectable droplets was greater for coarse droplets than medium droplets. Thus, a fine mist of droplets too small to be detected by the papers might still be drifting beyond the application area to affect non-target vegetation or contaminate water.

We observed evidence of drift at least 8 m away from the application area with both droplet sizes, under relatively low wind speeds (1.1 – 2.2 kph south-southwest). The sheltered channel where the targeted *P. australis* patch was located likely contributed to these low wind velocity measurements. Notably, wind measurements were recorded within a canoe around the base of the patch and may not have effectively captured the wind variation or gusts occurring

along the top of the canopy and did not reflect the air movements generated by the RPAS downwash from the rotors. In a similar study, Chen et al. (2020) set droplet collectors within the application area and along the downwind side of the flight path. With measured wind speeds of 7 – 9 km/h, they found medium droplets drifted 8 – 10 m downwind from the flight path. The difference in wind speed between our study and that by Chen et al. (2020) did not lead to a major difference in drift distance. Moreover, although the wind direction in our study was south-southwest, we did not observe notably greater drift along that axis. Combined, this suggests that although wind is typically considered the primary contributor to herbicide drift with aerial applications (Government of Canada, 2020), in the RPAS-based application at very low wind speeds, the force generated by the RPAS downwash may be more important than the effect of environmental wind.

In our study, a flight height of approximately 3 m was used for both droplet-size missions. RPAS-based applications have tested flight heights ranging from 1 – 6 m above the plant canopy (e.g., Huang & Thomson, 2011; Lan et al., 2021). Higher application heights have been associated with increased product drift (Huang & Thomson, 2011), which may explain differences in observed drift between our study (3 m above canopy) and that of Chen et al. (2020; 1.5 m above canopy). In fact, Dengeru et al. (2022) only observed drift within 5 m downwind of the flight center line when spraying fine droplets from a height of 1.6 m above the canopy. These differences suggest that herbicide droplet dynamics may differ when sprayed by an RPAS, with flight height being a greater contributor to droplet drift when differences in droplet size are minimal, due to the additional downward forces produced by the rotors. This is likely due to the RPAS being closer to the canopy and the rotor propulsion more directly influencing the path of droplets to the target foliage (Lan et al., 2021). While it seems that flying

as close to the canopy as possible has benefits for drift reduction, application height also impacts the effective swath width of each pass (Martin et al., 2019). Flying closer to the canopy can increase the number of passes required to treat the same area, influencing how much *P. australis* can be treated within one battery life and ultimately impacting treatment efficiency. Further, reducing the swath width with low flight heights may result in streaking in the application and missed *P. australis* ramets along the edge of each pass. Therefore, it is worthwhile to conduct additional studies to determine optimal flight heights for RPAS-based herbicide application on *P. australis*, that minimize drift and optimize the swath width or area of *P. australis* to be treated along a single pass.

While our study provides valuable insights into the droplet deposition of RPAS-based herbicide applications, it is important to acknowledge certain limitations. The use of water-sensitive papers to assess droplet deposition provided a quantitative measure of percent area coverage and VMD within the *P. australis* canopy. However, this approach has limitations in quantifying the spatial variability of herbicide deposition and distinguishing between overlapping droplets. Therefore, we were unable to assess the uniformity of deposition between droplet sizes or accurately quantify the number of droplets that intercepted each paper. Additionally, the droplet pattern observed on the water-sensitive papers is subject to a spread factor that is difficult to correct. The spread factor for droplets when using a water-only spray is roughly 2.0 for medium or coarse droplets (Hypro, 2006). However, inclusion of chemical surfactants, like that of Aquasurf® non-ionic spray adjuvant (Norac Concepts Inc., Pesticide Registration Number 32152) used in our study, alters the surface tension properties of the herbicide droplets to improve retention on the target foliage (Liu, 2004) and likely increases this spread factor. Thus,

the difference between the measured droplet stain and the actual size of the liquid droplet striking the paper is an overestimate of the true droplet size.

3.5 Conclusions

In this study, we report the droplet distribution and drift potential of medium and coarse herbicide droplets applied by an RPAS to a dense, homogenous *P. australis* canopy. Both medium and coarse droplet sizes achieved similar distribution throughout the canopy, suggesting that droplet size had minimal impact on canopy penetration. However, future research should aim to quantify ground losses from RPAS-based herbicide applications as downward forces from rotor propulsions can influence droplet penetration depth within the canopy. A greater decay rate of percent area coverage and volume median diameter (VMD) for medium droplets compared to coarse droplets suggests fewer medium droplets drifted away from our application area. Droplet drift was similar to values reported in other studies, with evidence of herbicide drift being observed at least 8 m away from the application area for both droplet sizes. These findings reveal that the consequences of droplet size choice are minimal between medium and coarse categories. We also highlight the importance of propulsion and RPAS downwash in herbicide drift from RPAS-based applications in the field, as well as the need for further research on potential interactions among flight parameters. While the use of water-sensitive papers allowed us to quantify the amount of herbicide that landed beyond the application area, there is a knowledge gap between herbicide droplet drift quantified in field studies and actual observed damage to surrounding vegetation. Understanding the threshold values for herbicide droplet deposition necessary to damage the target vegetation would allow us to effectively suppress the target species while minimizing non-target impact from lateral herbicide drift. By determining the

percent area coverage and VMD required to induce a toxic response in plants, we could refine herbicide application strategies to maximize efficacy and environmental safety. Future studies should aim to quantify the minimum effective herbicide dose for suppression of *P. australis* to better inform the consequences of herbicide drift rates to non-target vegetation. Additionally, quantifying ground losses from RPAS-based herbicide applications to *P. australis* under various flight parameters will help to optimize canopy penetration without excessive product wastage. Studies should employ repeated trials under consistent environmental conditions to more robustly evaluate the optimal parameters for RPAS-based herbicide application on *P. australis*.

4.0 Quantifying spray accuracy of targeted RPAS-based herbicide applications on *Phragmites australis*

4.1 Introduction

The use of herbicides to control invasions of *Phragmites australis* ssp. *australis* remains a necessary tool for many wetland managers (Hazelton et al., 2014). When applying herbicide to wetland ecosystems to suppress invasive *P. australis*, minimizing off-target effects on native plants is paramount in ensuring vegetation community recovery. Yet, this must be balanced against the risk of undertreatment, which leaves remnant *P. australis* in place to recolonize or requires repeated follow-up herbicide applications that are costly and risk herbicide accumulation in wetlands. Determining the accuracy of a given herbicide application method is necessary to refine application methods and select the appropriate method for the invasive plant management context. Unfortunately, limited information about the extent of off-target effects is available. For example, in an agricultural study, Tian (2002) developed a sensor-based herbicide application on a ground sprayer that was able to target and spray agricultural weeds with 91% accuracy; however, no data were reported on off-target depositions. In another study, Rasmussen et al. (2019) more explicitly investigated the spatial accuracy of ground-based herbicide spraying, contrasting planned spray polygons to the actual patch area sprayed. Despite a growing interest in application accuracy, similar work has not been published with respect to aerial herbicide applications, where the spatial footprint may extend well beyond the bounds of the application area.

Aerial herbicide applications to control invasive plants present a trade-off between overtreatment and undertreatment and in *P. australis* management, this is further exacerbated by

the non-uniform distribution of *P. australis* stands. At the centre of a *P. australis* patch, the ramets are tightly clustered together, forming a monoculture (Tulbure et al., 2007). However, in unconstrained wetland environments, *P. australis* patches expand from their margins with leading rhizomes, such that *P. australis* at the patch margins tends to grow at lower density and patches are irregularly shaped (Jung et al., 2017). Using aerial application methods to treat these patch margins thus results in overtreatment and off-target effects on co-occurring native plants. Unfortunately, in *P. australis* management, undertreatment may pose as much of a threat as overtreatment. As *P. australis* has a high capability to expand its range, remnant ramets that evade initial herbicide application have been found to multiply by a factor of seven in one growing season (Robichaud & Rooney, 2021a). For that reason, treating all *P. australis* ramets in an intended treatment area is necessary to minimize re-establishment and the need for follow-up treatments in subsequent years. Reducing the amount of herbicide application required in follow-up treatments also addresses concerns about the cumulative effects of repeated herbicide applications (Crowe et al., 2011) and the risk of herbicide-resistance developing in *P. australis* (Government of Ontario, 2001).

Since the approval for over-water herbicide application to suppress invasive *P. australis* in Canada, aerial herbicide application has become a useful tool, particularly for large invasions in difficult to access wetland environments. For example, a pilot project conducted by the Ontario Ministry of Natural Resources and Forestry begun in 2016, used helicopter application of a glyphosate-based herbicide under an Emergency Use Registration to suppress *P. australis* in coastal wetlands in Long Point Provincial Park and Rondeau Provincial Park: two biodiversity hotspots with high densities of species at risk (Ministry of Natural Resources and Forestry, 2016). Observations made during the Emergency Use Registration *P. australis* suppression pilot

project identified that the treatments, though highly effective in reducing the extent of *P. australis* (Robichaud & Rooney, 2021a), and promoting the eventual recovery of native vegetation (Jordan, 2022), initially resulted in extensive secondary invasions (Robichaud & Rooney, 2021a). Ministry staff remarked on the large area of native vegetation surrounding treated patches of *P. australis* that was affected by the herbicide application (Adam Hogg, Ministry of Natural Resources and Forestry, Peterborough, Ontario, pers. comm.), though this was not quantified. It is possible that the recovery of native vegetation would have been expedited if the footprint of herbicide application had been more precisely restricted to the extent of *P. australis*, minimizing off-target effects (Boutin et al., 2013). Remotely piloted aircraft systems (RPASs) may offer an alternative means of aerial application that reduces the extent of off-target effects by providing a more accurate and precise application of herbicides.

Importantly, current Canadian regulations only permit aerial application of herbicide to control *P. australis* over standing water with the use of a helicopter (Pest Management Regulatory Agency, 2020). The finest grain control on aerial application footprint of an herbicide is limited by the application method's swath width. Under typical helicopter application flight conditions (10 m above a canopy and travel at roughly 20 m/s), the effective swath width can range between 25 – 30 m, depending on the nozzle type used (Yao et al., 2021). Consequently, to capture all *P. australis* ramets in an application area where patches are smaller or discontinuous, considerable overtreatment is unavoidable. Helicopter-based application is thus best suited to treating large (> 1 ha) monocultures of *P. australis*.

Though not currently approved for use over standing water to control *P. australis* in Canada, RPASs can fly much closer to the *P. australis* canopy (~3 m above the canopy) and at lower flight speeds (2 m/s), resulting in swath widths about an order of magnitude smaller than

by helicopter application (approximately 3 m; Adrian Rivard, pers. comm.). Therefore, when navigating the edges of small, irregularly shaped *P. australis* patches, RPAS-based herbicide application could potentially reduce the extent of overtreatment by up to 90%. Further, the lower flight heights achievable with RPAS application will likely decrease herbicide drift (Lan et al., 2021). RPAS-based herbicide application has the potential to optimize the trade-off between undertreatment and overtreatment and reduce off-target effects, expediting native plant community recovery after *P. australis* suppression. However, a site-level review of the accuracy of this technology and the resulting spatial footprint of the herbicide application are necessary to test the extent of off-target effects and drift in a realistic setting, which regulators can use in evaluating the safety of RPAS-based herbicide application.

In this chapter, we compare remotely-sensed images of *P. australis* invasions, before and after herbicide application by an RPAS, at four different sites in provincially significant wetlands in southern Ontario, representing model environments to test this technology. Our objectives were: 1) to quantify application accuracy of the RPAS-based herbicide application across a range of real-world conditions and 2) to evaluate any post-treatment effects of the herbicide application. Application accuracy was characterized by on-target damage, undertreatment, and intended overtreatment areas. We define on-target damage as the agreement between where *P. australis* was identified in the pre-treatment remote-sensing image classification and the intended treatment area exported by the RPAS flight paths, we define undertreatment as areas where the intended treatment areas do not fully cover the *P. australis* patch as delineated in the pre-treatment classification, and we define intended overtreatment as areas where the intended treatment areas extend beyond the border of the *P. australis* patch delineated in the pre-treatment classification. Post-treatment effects were characterized by non-target plant damage and

evidence of herbicide drift. We define non-target plant damage as areas where vegetation that was not *P. australis* in the pre-treatment classification are classified as damaged from the herbicide treatment in the post-treatment classification. We define drift as classified vegetation damage in the post-treatment classification that occurred outside the bounds of the intended treatment areas.

4.2 Methods

4.2.1 Study design

We completed a before and after comparison of remotely-sensed imagery at four Provincially Significant Coastal Wetlands in Ontario (Fig. 4-1), with imagery collected during peak aboveground biomass (July) of the summer before and the summer after RPAS-based herbicide application. Combined with the RPAS flight paths during the herbicide application, we were able to quantify the spatial extent of undertreatment, intended overtreatment, and extent of spray drift. At two of the sites, we used point-intercept transect surveys along the edges of *P. australis* patches to detect subtler signs of herbicide damage to native vegetation as evidence of overtreatment and any remnant *P. australis* ramets as evidence of undertreatment.



Figure 4-1. Map of four Provincially Significant Wetlands used as pilot sites for RPAS-based applications of Habitat® Aqua to invasive *Phragmites australis*.

4.2.2 Field sites

In addition to Rondeau Provincial Park and Baie du Doré (described in Chapter 2.0), two other provincially significant wetlands, Spongy Lake and Wood Drive, were imaged as part of a site-level analysis of the RPAS-based herbicide application (Fig. 4-1). Spongy Lake is a peatland in a kettle lake depression, owned by the University of Waterloo, found east of the town of Baden, Ontario. The development of peatland in the basin is a product of lake succession which has caused the lake to fill in with organic matter, creating a base that is unable to support the weight of a person equipped with a backpack sprayer, but is too thick to allow the passage of a

boat. As the invasion of *P. australis* at Spongy Lake is relatively small, herbicides cannot be sprayed by a helicopter or airplane without considerable negative impact to the many rare plant species in the wetland. Additionally, the inaccessibility of the site prevents treatment by backpack spraying or amphibious vehicles.

Wood Drive coastal wetland is a swamp-marsh complex located on the eastern shore of Lake Huron. This complex hosts several species at risk turtles, snakes, and birds as well as rare marsh vegetation (Gilbert, 2017), yet is orphaned land with no clear ownership and consequently, no individual responsible for its management. The wetland has been split into three different blocks to facilitate *P. australis* suppression. The center “block” (Gilbert, 2017) was selected for treatment in this study and contained nearly 34 ha of dense *P. australis* in 2013. Efforts to control *P. australis* at this site occurred in 2014 (herbicide treatment) and 2015 (herbicide treatment paired with burning and rolling the standing dead biomass) in areas that lack standing water. However, as Lake Huron’s water levels increased nearly one meter above the long-term average between 2015 and 2020 (National Oceanic and Atmospheric Administration, 2024), the areas permitted for herbicide treatment were limited due to restrictions on over-water herbicide application in Canada. Additional herbicide treatments (backpack spraying) occurred near the RPAS-based herbicide application areas at Wood Drive in 2022; impeding our ability to distinguish overtreatment or drift due to the RPAS-based application from treatment by backpack spraying. Consequently, we excluded Wood Drive from our post-treatment classification of damaged vegetation.

4.2.3 Aerial imagery

High resolution (~1.5 cm/pixel ground sampling distance) aerial images were captured by Ontario Power Generation's Advanced Inspection and Maintenance team at the four study sites in July 2022, under clear weather conditions. We conducted flights at altitudes ranging from 100-120 m above ground level to optimize image resolution and coverage. Details regarding the RPAS units, cameras, and image specifications for each site can be found in Table 4-1. To ensure consistency across years, flight paths were generated in WingtraHub or the DJI Pilot App (RPAS model dependent) and were used to replicate the flights in July 2023 to capture the same extent of the sites after the herbicide treatment.

The Advanced Inspection and Maintenance team georeferenced the images using the real-time kinematic global navigation satellite systems-based land survey technique. This method uses high-precision global navigation satellite system receivers to establish accurate ground control points across the study area, providing centimeter-level positioning accuracy and ensuring precise spatial referencing of the aerial images (de Queiroz et al., 2022). Differential corrections were applied in real-time to correct for atmospheric errors and satellite orbit deviations, increasing the overall georeferencing accuracy of the ground control points.

Following georeferencing, the aerial images were processed using Pix4Dmapper software (Prilly, Switzerland). Pix4Dmapper is a photogrammetry tool designed for processing RPAS imagery and generating accurate orthomosaics, among other geospatial models. The software completes both radiometric and atmospheric corrections on the images to correct for any lens distortions, atmospheric effects, and radiometric inconsistencies (Pix4D, 2017). The images are then stitched through a process called mosaicking to create a single orthomosaic of each study site.

Table 4-1. Summary of high-resolution imagery collection including collection date, RPAS model, camera used, image resolution, and overlap percentage at each study site.

	Site	Image collection date	RPAS model	Camera	Image resolution	Overlap percentage
Pre-treatment	Baie du Doré	July 6, 2022	WingtraOne Gen I	Sony RX1R II 42MP	1.30 cm/pixel ground sampling distance (GSD)	85 Side 81 Frontal
	Rondeau Park	July 19, 2022	WingtraOne Gen I	Sony RX1R II 42MP	1.60 cm/pixel GSD	85 Side 85 Frontal
	Spongy Lake	July 20, 2022	DJI Matrice 300 RTK	Zenmuse P1 45MP with 35mm lens	1.51 cm/pixel GSD	85 Side 80 Frontal
	Wood Drive	July 18, 2022	DJI Matrice 300 RTK	Zenmuse P1 45MP with 35mm lens	1.26 cm/pixel GSD	85 Side 80 Frontal
Post-treatment	Baie du Doré	July 7, 2023	WingtraOne Gen II	Sony RX1R II 42MP	1.53 cm/pixel GSD	85 Side 70 Frontal
	Rondeau Park	July 19, 2023	WingtraOne Gen II	Sony RX1R II 42MP	1.65 cm/pixel GSD	85 Side 85 Frontal
	Spongy Lake	July 20, 2023	DJI Matrice 300 RTK	Zenmuse P1 45MP with 35mm lens	1.71 cm/pixel GSD	85 Side 80 Frontal
	Wood Drive	July 18, 2023	DJI Matrice 300 RTK	Zenmuse P1 45MP with 35mm lens	1.26 cm/pixel GSD	85 Side 80 Frontal

4.2.4 RPAS-based herbicide application

RPAS-based application of Habitat® Aqua was completed as described in Chapter 2.0. In accordance with the Research Authorization 0009-RA-22 issued by Health Canada to BASF, a maximum of 2 ha of *P. australis* were treated at each of the sites. Within the herbicide application allowances, site managers provided input on areas of *P. australis* to target with the RPAS-based treatment. Spongy Lake and Baie du Doré had two treatment areas and Rondeau Provincial Park and Wood Drive had three treatment areas. Baie du Doré and Rondeau Provincial Park also had control areas that were left untreated to support on-the-ground vegetation surveys (see section 4.2.7 “Vegetation surveys”). Table 4-2 details weather parameters at the time of each herbicide application.

Table 4-2. Treatment dates and weather parameters during the RPAS-based herbicide application at each of the four pilot sites. Temperature and relative humidity were measured with an Extech RH300 hygro-thermometer (Extech Instruments, Waltham, MA, USA). Wind direction and wind velocity were measured with a Kestrel 1000 anemometer (Kestrel Instruments, Boothwyn, PA, USA).

Site	Date of treatment	Temperature (°C)	Relative humidity (%)	Wind direction	Wind velocity (kph)
Spongy Lake	August 31, 2022	26.0	54.7	West	1.1
Baie du Doré	September 1, 2022	25.0	53.6	West	1.7
Rondeau Provincial Park	September 14, 2022	23.0	63.4	South	5.0
Wood Drive	September 16, 2022	29.0	62.9	South-west	1.0

4.2.5 Image classification

To accurately map the extent of *P. australis* before treatment and assess the subsequent plant damage post-treatment, we classified the remotely-sensed images with manual delineation. We selected manual delineation over other classification techniques (e.g., pixel- or object-based) based on several factors, including the extremely high resolution of the images, relatively small geographic regions (~30 ha) to be classified, and few classes (live *P. australis* and damaged vegetation from herbicide treatment) we were interested in mapping. Manual delineation was also found to offer the highest classification accuracy for *P. australis* in previous research by Marcaccio (2016). Across both years, a single interpreter classified all sets of images using ArcGIS Pro (v. 3.1.0, ESRI, Inc., Redlands, CA) to maintain consistency and reproducibility in the classifications. This approach minimized potential variability in classification outcomes and ensured reliable comparisons between pre-treatment and post-treatment classifications. The interpreter's participation in the vegetation surveys at Rondeau Provincial Park and Baie du Doré (see section 4.2.7 "Vegetation surveys"), provided context to the layout of the sites and locations of *P. australis* patches, prior to classifying the images. We recorded 100 GPS coordinates in the field across both surveyed sites to confirm the accuracy of the image classification. Coordinates were randomly distributed along the boundaries of *P. australis* patches (pre-treatment), and in dead vegetation (post-treatment) to serve as ground truthing points.

4.2.6 Spatial analyses

Spatial overlay analysis

Log files capture and record the geographic location of the spray-equipped RPAS during its herbicide-application flight. Based on the calibrated swath width (3 m) at the RPASs target flight height above the *P. australis* canopy (Adrian Rivard, pers. comm.), we added a 1.5 m wide

buffer to the flight lines in the log files from the RPAS to create a polygon of the intended treatment area. During the automated flights, the RPAS sprayed along each flight line, shutting off when turning and shifting lines (Fig. 4-2). Due to pilot concerns about adequately applying herbicide to the perimeter of the delineated *P. australis* patches, a semi-assisted flight to treat the patch perimeter was completed after each initial automated flight (Fig. 4-2; Adrian Rivard, pers. comm.). During semi-assisted flights, the log files track the path taken by the RPAS, but do not record whether the RPAS is actively spraying or not. Through consultation with the pilot, the beginning and end of active spraying during the semi-assisted flights was estimated.

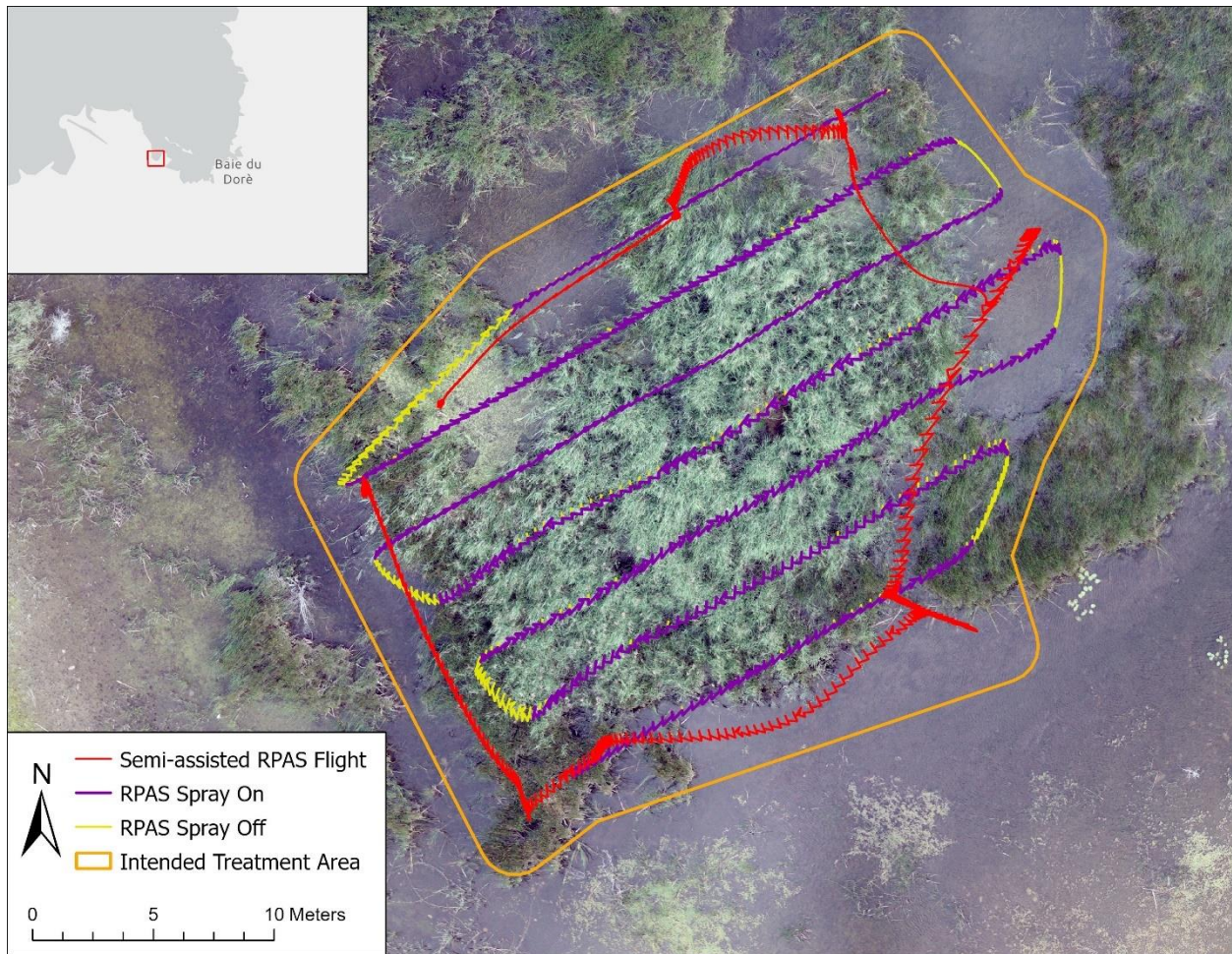


Figure 4-2. Map of semi-assisted flight lines (red; spraying herbicide along the ferry lines), automated flight lines (purple; spraying herbicide), and ferry lines (yellow; not spraying herbicide), used in the RPAS-based Habitat® Aqua application to the intended treatment area (orange polygon). Map depicts the treatment polygon ID “BDD-South”. Basemap imagery was collected by Ontario Power Generation’s Advanced Inspection and Maintenance team in 2022.

To quantify application accuracy, we intersected classified live *P. australis* areas with their corresponding intended treatment areas from the RPAS-based herbicide application (Fig. 4-3A). To quantify undertreatment areas, we overlaid the intended treatment area and the pre-treatment *P. australis* classification and removed the areas that were coincident (Fig. 4-3B). To quantify intended overtreatment areas, we overlaid the pre-treatment *P. australis* classification and intended treatment area and removed the areas that were coincident (Fig. 4-3C). To quantify non-target plant damage, we overlaid the post-treatment dead vegetation classification and pre-

treatment *P. australis* classification and removed areas that were coincident (Fig. 4-3D). To quantify drift, we overlaid the post-treatment dead vegetation classification and their corresponding intended treatment areas and removed the areas that were coincident (Fig 4-3E). All spatial overlays were completed in ArcGIS Pro (v. 3.1.0, ESRI Inc., Redlands, CA) and specific overlay functions are summarized in Table 4-3. In ArcGIS Pro, we extracted the area values for each of the outputs (on-target, under- and intended overtreatment areas, non-target plant damage, and drift) from the overlay analysis in the projected coordinate system North American Datum (NAD) 1983, Universal Transverse Mercator (UTM) Zone 17 North. The UTM projection provides a conformal mapping the of Earth's surface onto a two-dimensional Cartesian coordinate system, allowing accurate measurements of area, distance, and angles within the zone (Thomas, 2017). Using the area values, we calculated the percentage of each output type in relation to the area of the targeted *P. australis* patch, as determined in the pre-treatment remote-sensing image classification.

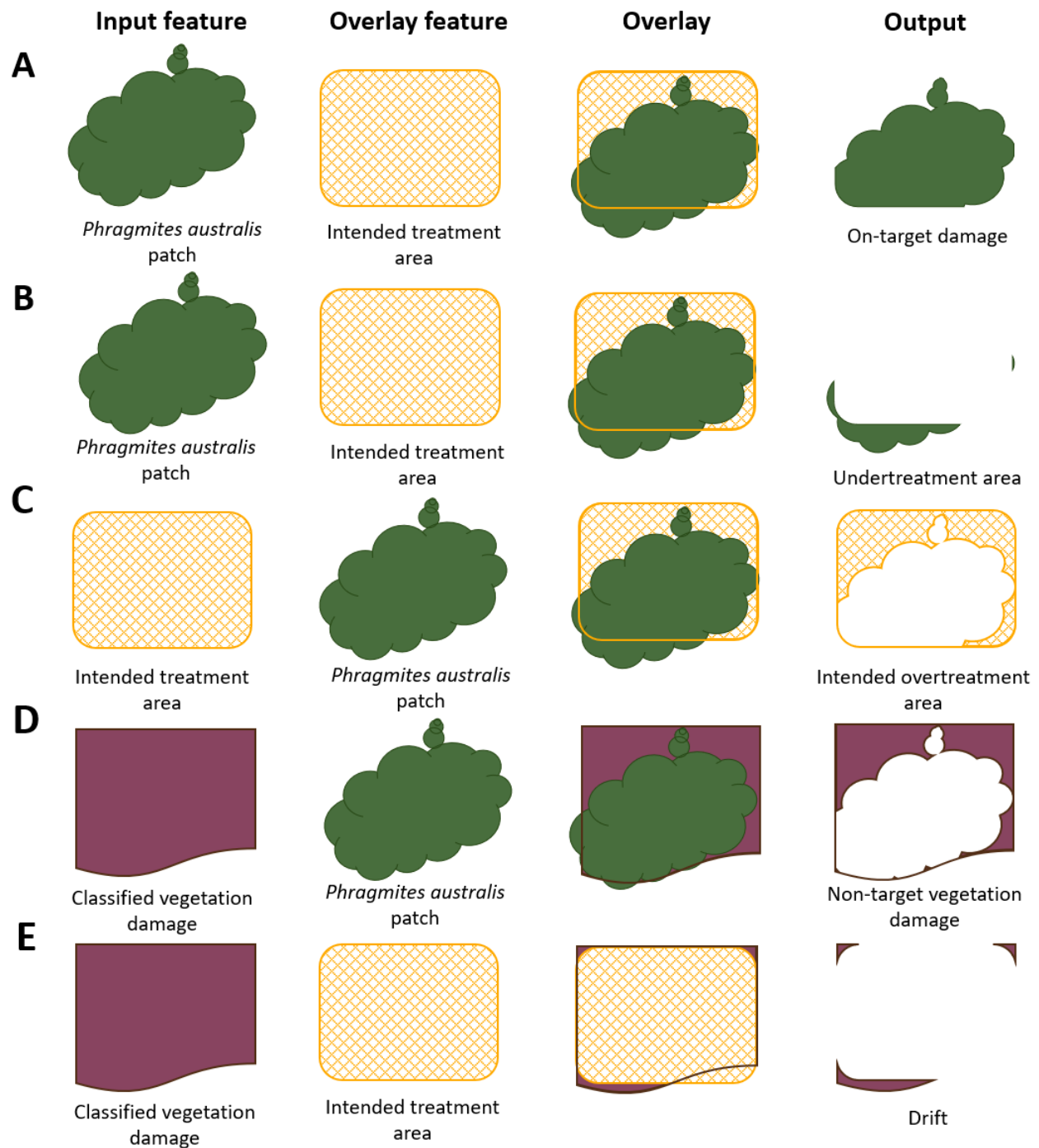


Figure 4-3. Graphic of input and overlay features used for on-target damage (A), undertreatment (B), intended overtreatment (C), non-target vegetation damage (D), and drift (E) area outputs. Green polygon represents a digitized *Phragmites australis* patch, orange rectangle represents an intended treatment area, and purple polygon represents digitized vegetation damage from the post-treatment image classification, which combines intended overtreatment and drift.

Table 4-3. Summary of overlay function types, associated input and overlay features, and the resultant output polygons produced by the overlay function.

Overlay type	Input feature	Overlay feature	Output
Intersect	Pre-treatment <i>P. australis</i> classification	Intended treatment area	On-target damage
Erase	Pre-treatment <i>P. australis</i> classification	Intended treatment area	Undertreatment
Erase	Intended treatment area	Pre-treatment <i>P. australis</i> classification	Intended overtreatment
Erase	Post-treatment dead vegetation classification	Pre-treatment <i>P. australis</i> classification	Non-target vegetation damage
Erase	Post-treatment dead vegetation classification	Intended treatment area	Drift

Drift analysis

To assess drift more comprehensively, we also calculated drift in relation to the area sprayed with herbicide by the RPAS, to eliminate bias if larger intended treatment areas result in a larger extent of drift. We also calculated the perimeter-area ratio of each intended treatment area to provide insight on the proportion of edge available for drift to occur along, relative to the size of the intended treatment area. To assess the quantity of drift observed around the application areas from the RPAS-based herbicide application, we calculated the percentage of the intended treatment area's perimeter that drift appeared along. We extracted the perimeter of the intended treatment area in the projected coordinate system NAD 1983, UTM Zone 17 North. To measure the length of perimeter that drift appeared along, we used the measure distance tool (ESRI, n.d.-b) in ArcGIS Pro to trace the intersecting boundary between the intended treatment area and outputted drift polygon. To ensure the entire intersecting boundary was measured, we

enabled the snapping drawing aid (ESRI, n.d.-c) to capture all vertices along the drift polygon as the measure distance tool recorded the distance.

To quantify the extent of drift away from the application area, we measured the maximum distance at which vegetation damage was observed away from each application area. We took measurements perpendicular to the edge of the intended treatment area where the greatest distance of drift was seen. Using the feature vertices to point tool (ESRI, n.d.-a), we established endpoints of the drift polygon. Subsequently, we measured the distance from a vertex point to the edge of the intended treatment area using the measure direction distance tool (ESRI, n.d.-b), ensuring that the angle of intersection with the intended treatment area's boundary was set at 90°. We selected and measured the distance of five vertices considered to be the furthest instances of drift for each application area. From the five extracted values, we recorded the highest as the maximum drift distance. We also noted the dominant wind direction for each site during application as west for Spongy Lake and Baie du Doré, south for Rondeau Provincial Park, and south-west for Wood Drive.

4.2.7 Vegetation surveys

In August 2022, we conducted baseline vegetation surveys at Baie du Doré and Rondeau Provincial Park wetlands, prior to the herbicide application. We deployed 10 m long transects (N=10 at Baie du Doré; N=20 at Rondeau Park) passing through the edge of dense (> 20 live stems/m²) *P. australis* patches into the surrounding vegetation. We set up the transects in a paired manner, so that half were established in the control areas, and the other half in the proposed treatment areas to be sprayed with Habitat® Aqua by the RPAS. We marked the beginning and end of each transect by placing a bamboo stake with flagged labels into the

sediment and recorded a GPS coordinate with sub-meter accuracy (SXPro GNSS, Geneq Inc., Montreal, QC, Canada) at both ends to facilitate locating the transect in the following year. We took measurements every 20 cm following point-intercept sampling (Madsen, 1999) to capture the boundary between the *P. australis* patch and the surrounding vascular plant species along the transects. We also recorded water depth and canopy height at each 20 cm interval. In August 2023, we conducted follow-up vegetation surveys at both wetlands following the same sampling methods to assess the post-treatment state of the transects.

To determine the extent of remnant live *P. australis* along each post-treatment transect, we measured the distance between the first- and last-intercepted live *P. australis* ramet on each transect. Using the GPS locations of the transects starting and ending locations taken in the field, we overlaid the intended treatment areas to assess the portion of each transect that was sprayed with herbicide. Where transects extended beyond the boundary of the intended treatment area, we omitted intercepted live *P. australis* ramets from the total distance as they were not treated with the herbicide.

4.3 Results

4.3.1 Site factors and analysis of the RPAS-based herbicide treatment areas

Spongy Lake had 0.25 ha of live *P. australis* targeted for treatment, broken into two treatment areas. The polygons were 0.21 ha and 0.35 ha in area and had perimeter-area ratios of 0.08 and 0.09. The lake is bordered by deciduous trees and the *P. australis* patches have little vegetation directly surrounding them. Baie du Doré had 0.13 ha of live *P. australis* targeted for treatment, broken into two treatment areas. The polygons were 0.07 ha and 0.53 ha in area and had perimeter-area ratios of 0.06 and 0.15. The site was mainly open water marsh and the

targeted *P. australis* patches were surrounded principally by *Typha x glauca* (Hybrid Cattail), floating, and submersed aquatic vegetation. Rondeau Provincial Park had 0.46 ha of live *P. australis* targeted for treatment, across three treatment areas. The polygons ranged 0.10 – 0.57 ha in area and had perimeter-area ratios between 0.06 – 0.16. The treatment site was a swamp slough with *P. australis* patches bordered by deciduous trees. Wood Drive had 0.76 ha of live *P. australis* targeted for herbicide treatment, across three treatment areas. The polygons were between 0.35 – 0.89 ha in area and had perimeter-area ratios between 0.05 – 0.08. The site was a coastal marsh with abundant stands of *Typha x glauca* interspersed and surrounding the patches of *P. australis*.

4.3.2 Spatial overlay analysis: assessment of RPAS-based herbicide application accuracy on targeted areas of *Phragmites australis*

Results from our overlay analysis revealed the spatial footprint of the RPAS-based herbicide application in relation to the targeted areas of *P. australis*. Measurements quantifying the application accuracy (e.g., on-target damage, undertreatment area, and intended overtreatment area) and post-treatment effects (e.g., non-target vegetation damage area and drift as a percentage of the *P. australis* area) for each intended treatment area are provided in Table 4-4. Spongy Lake and Baie du Doré both had two polygons of *P. australis* targeted for treatment, while Rondeau and Wood Drive had three polygons. Across all intended treatment areas, the average percentage of on-target damage was between 70 – 100% (average = 91%; std. = 10%). The average undertreatment area across all intended treatment areas varied from 0 – 30% (average = 9%; std. = 10%) of the targeted *P. australis* area. Across all sites, the average amount of intended overtreatment was between 74 – 440% (average = 172%; std. = 106%) of the targeted *P. australis* area.

We assessed the post-treatment effects of the RPAS-based herbicide application with two additional parameters, non-target plant damage and drift. Across Spongy Lake, Baie du Doré, and Rondeau Provincial Park, the average amount of non-target plant damage varied from 42 – 229% (average = 102%; std. = 66%) of the targeted *P. australis* area. The average amount of drift observed across Spongy Lake, Baie du Doré and Rondeau Provincial Park was between 4 – 34% (average = 14%; std. = 10%) of the targeted *P. australis* area.

Table 4-4. Summary of RPAS-based herbicide application accuracy outputs from spatial overlay analysis. Output values are reported as percentages of pre-treatment *Phragmites australis* area. Data were collected from Spongy Lake, Baie du Doré, Rondeau Provincial Park, and Wood Drive wetlands. Non-target plant damage and drift were not calculated for Wood Drive due to the inability to complete the post-treatment classification of damaged vegetation attributed to the RPAS-based herbicide application.

Site	Treatment polygon ID	Intended treatment area (m ²)	Pre-treatment <i>P. australis</i> area (m ²)	On-target damage (%)	Undertreatment area (%)	Intended overtreatment area (%)	Non-target plant damage area (%)	Drift (%)
Spongy Lake	SL-North	3464.65	1608.48	95	5	120	113	34
	SL-South	2131.51	918.57	98	2	130	65	15
Baie du Doré	BDD-North	5332.24	983.57	99	1	440	229	10
	BDD-South	723.60	314.04	100	0	130	70	14
Rondeau Provincial Park	RPP-North	5723.08	3651.77	83	17	74	42	13
	RPP-Centre	1373.75	451.86	85	15	219	140	9
	RPP-South	993.73	504.94	86	14	110	56	4
Wood Drive	WD-North	7177.60	3244.16	99	1	122	N/A	N/A
	WD-Middle	3516.86	1692.75	70	30	138	N/A	N/A
	WD-South	8908.77	2690.03	96	4	235	N/A	N/A

4.3.3 Drift analysis: quantifying drift from RPAS-based herbicide application outside of application area

Drift occurred outside of all seven intended treatment areas, at the three sites where post-treatment identification of plant damage was possible (Spongy Lake, Baie du Doré, and Rondeau Provincial Park). Due to the high rates of intended overtreatment observed from the herbicide treatment in section 4.3.2, drift was not well represented by its percentage in relation to the total area of *P. australis* that was targeted for RPAS-based herbicide application at each wetland. Instead, we consider the percentage of observed drift in relation to the area of each intended treatment area it occurred around, with two or more areas receiving treatment per wetland. Measurements characterizing the intended treatment areas (e.g., total area and perimeter to area ratio) and measurements quantifying the extent of drift around each intended treatment area (e.g., drift area, drift area as a percentage of sprayed area, or drift as a percentage of intended treatment area perimeter) are provided in Table 4-5.

Spongy Lake and Baie du Doré both had two polygons of *P. australis* targeted for treatment, while Rondeau had three polygons. In general, the intended treatment areas were small (< 0.6 ha), with smaller intended treatment areas having a larger perimeter to area ratios. The extent of drift varied from 2 – 16% (average = 6%; std. = 5%) of the total treatment area and was neither strongly related to the area nor perimeter of the intended treatment area. The extent of drift was more consistently about a quarter of the intended treatment area perimeter (average = 24%; std. = 11%). The distance away from the intended treatment area to which drift could be detected ranged from about 3 – 20 m (average = 8 m; std. = 6 m).

Table 4-5. Summary of drift (evidence of vegetation death beyond the application area) after RPAS-based herbicide application. Data were collected from Spongy Lake, Baie du Doré, and Rondeau Provincial Park wetlands.

Site	Treatment polygon ID	Intended treatment area (m ²)	Perimeter-area ratio of intended treatment area	Drift area (m ²)	Intended treatment area perimeter (m)	Perimeter of shared boundary between intended treatment area and drift polygon (m)	Drift percentage by intended treatment area (%)	Drift percentage by perimeter (%)	Maximum distance of drift (m)
Spongy Lake	SL-North	3464.65	0.08	547.56	279.34	70.75	16	25	20.44
	SL-South	2131.51	0.09	138.21	182.27	47.23	6	26	6.83
Baie du Doré	BDD-North	5332.24	0.06	98.52	305.02	49.02	2	16	8.11
	BDD-South	723.60	0.15	43.75	105.10	21.43	6	20	4.35
Rondeau Provincial Park	RPP-North	5723.08	0.06	489.40	347.28	163.63	9	47	8.99
	RPP-Centre	1373.75	0.13	42.30	185.23	37.16	3	20	4.38
	RPP-South	993.73	0.16	20.46	157.61	20.21	2	13	2.94

4.3.4 Analysis of field-collected transect data

We intercepted live *P. australis* ramets in the treatment areas along six of the 15 post-treatment transects we measured (40%); three at Baie du Doré and three at Rondeau Provincial Park. Among all treatment transects there was a total of 15 intercepts of live *P. australis*, representing 2% of possible intercepts on all post-herbicide application treatment transects. The number of live *P. australis* intercepts ranged from one to five per transect, with the average number of intercepts being two to three, out of a possible 50. The average distance between the two most extreme remnant *P. australis* ramets on the post-treatment transects was 190 cm (std. = 224 cm), ranging from 0 cm to 480 cm. By measuring canopy height at each of the intercepts pre- and post-treatment, we were able to confirm that the intercepted live *P. australis* survived the herbicide application and did not arise from seed as *P. australis* ramets from seed has a shorter stature and less robust morphology compared to ramets from rhizome. Table 4-6 details the six post-treatment transects that live *P. australis* was intercepted on.

Table 4-6. Summary of live *Phragmites australis* intercepts on treatment transects in Baie du Doré and Rondeau Provincial Park post RPAS-based herbicide application.

Site	Transect ID	Number of live <i>P. australis</i> intercepts	Intercept distance from start of transect (cm)	Distance between live <i>P. australis</i> intercepts (cm)
Baie du Doré	BDDT01	3	200, 220, 240	40
	BDDT03	2	0, 480	480
	BDDT05	1	200	0
Rondeau Provincial Park	RPPT04	2	420, 480	60
	RPPT07	5	80, 140, 160, 440, 560	480
	RPPT10	2	820, 920	100

4.4 Discussion

Our study assessed the spatial footprint of RPAS-based herbicide applications to invasive *P. australis* in wetland environments. We aimed to evaluate the application accuracy and post-treatment effects of this application method. The on-target application rate averaged 91% of the *P. australis* area across all intended treatment areas, aligning with the on-target rates observed with ground-based patch spraying (Rasmussen et al., 2019). Intended overtreatment was evident, with nine out of ten of intended treatment areas exceeding the *P. australis* area by at least 100%. This was partly attributed to challenges in accurately identifying *P. australis* among other tall emergent vegetation like *Typha x glauca* (Hybrid Cattail) when creating application areas, but it also represents a trade-off between capturing all *P. australis* ramets in a treatment area and minimizing non-target plant damage. Similarly, in terms of post-treatment effects, non-target vegetation damage exceeded 100% of the *P. australis* area for most intended treatment areas, particularly where non-target emergent vegetation (as opposed to open water) bordered the *P. australis* patches. This trade-off is critical as any surviving *P. australis* will threaten long-term suppression success, but overtreatment harms the very plants we rely on to recolonize the treated areas and may delay the recovery of the vegetation community. Herbicide drift was variable, but the maximum observed extent of drift (~20 m perpendicular to the intended treatment area boundary) aligns with other recommendations from studies on buffer distances for RPAS-based pesticide application (Gibbs et al., 2021) and is considerably smaller than the maximum extent of drift from helicopter application. Likely, drift was exacerbated by using semi-assisted flights to capture the edges of each *P. australis* patch. With refinements of RPAS flight parameters along patch edges, drift may be reduced.

Our findings indicate that RPAS technology can effectively target herbicides to *P. australis* patches with an average on-target application rate of 91%. The on-target application rate aligns

closely to the 89 – 96% reported for ground-based patch spraying (Rasmussen et al., 2019), when the boom width was 3 m (in line with the swath width from our RPAS application). Several site-specific factors may have influenced the observed variance in RPAS-based application accuracy among our sites. At Rondeau Provincial Park, trees bordering the swamp slough hampered RPAS access along the boundary of certain treatment areas. In contrast, Baie du Doré's open marsh provided an unobstructed area for the RPAS to fly for each mission. Wood Drive had the largest area of *P. australis*, comprising mainly small and irregularly dispersed patches of *P. australis* with varying live stem density. The spatial distribution of the patches posed challenges in creating intended treatment areas without cutting off part of a *P. australis* patch or leaving remnant ramets near the edges. Whereas at Spongy Lake, the *P. australis* invasions were clustered in distinct patches at opposite ends of the lake, simplifying the delineation of the treatment area for each patch.

Intended overtreatment values were generally higher than expected, with only one of ten intended treatment areas having a total treated area less than double the footprint of *P. australis* targeted for treatment (Appendix A). An important factor in the extent of intended overtreatment is the distribution and shape of *P. australis* stands within each wetland and the logistical constraints of grouping these stands into polygons targeted for treatment and planning the flight paths to cover these polygons. Sites like Baie du Doré and Wood Drive where *P. australis* grew in a more heterogenous and patchy conformation will present a greater planning challenge and will necessitate a greater degree of intended overtreatment to mitigate undertreatment. It can be challenging to differentiate *P. australis* from other tall emergent vegetation, especially where *P. australis* stem density is thin at the patch margins. At two of our study sites, *Typha x glauca* (Hybrid Cattail) surrounded (Baie du Doré) or was interspersed within (Wood Drive) *P. australis*

patches, making it difficult to target *P. australis* exclusively. Herbicide applicators forced to choose between overtreatment and undertreatment often favour overtreatment as regrowth of surviving *P. australis* necessitates costly and extensive follow-up treatments. For example, Robichaud and Rooney (2021a) found that remnant *P. australis* increased live stem density seven-fold in a single year after herbicide treatment. Not only do follow-up treatments increase project costs and delay native plant community recovery, but they can lead to accumulation of herbicide residues in wetlands (Breckels & Kilgour, 2018) and could create conditions that favour the evolution of herbicide resistance in *P. australis* (Government of Ontario, 2001). Intended treatment areas with the greatest intended overtreatment rates also had the lowest undertreatment rates, showcasing this trade-off. For example, at Baie du Doré one of the treatment areas had < 1% of undertreatment area, but over 400% intended overtreatment area. Conversely, at Rondeau Provincial Park, the only intended treatment area to have < 100% intended overtreatment (i.e. the area of intended overtreatment was equivalent to the area of *P. australis* targeted for treatment) had nearly 17% of the *P. australis* patch left untreated. It is important to note that the amount of intended overtreatment is attributable to flight planning and the subjective nature of creating the intended treatment areas is a large contributor to the error observed. Additional training for RPAS pilots may improve the rates of intended overtreatment, however, some amount will likely be unavoidable when minimizing the potential of undertreatment area.

While intended overtreatment refers to the situation where the intended treatment area extended beyond the border of the *P. australis* patch delineated in the pre-treatment classification, non-target plant damage refers to the case where vegetation other than *P. australis* is classified as damaged by the herbicide treatment in the post-treatment classification. On

average, non-target plant damage was up to an additional 100% of the targeted *P. australis* area. The extent of non-target plant damage observed in this study can be attributed to variations in the spatial distribution of vegetation in relation to the *P. australis* patches at each intended treatment area. In some cases, high non-target plant damage was related to the intended overtreatment area within a given treatment area. This was the case at Baie du Doré where the large proportion of intended overtreatment encompassed nearby *Typha x glauca*, and as a result, the non-target plant damage was high (nearly 230% of the targeted *P. australis* area). However, when *P. australis* was surrounded primarily by water, or other vegetation was interspersed within the targeted *P. australis* area, intended overtreatment would be high, but non-target plant damage was relatively lower. This was evident at one of the intended treatment areas at Spongy Lake where intended overtreatment was 130% of the targeted *P. australis* area, but non-target damage was only 65%. Submersed aquatic vegetation in the surrounding water may not be as sensitive to Habitat® Aqua as the herbicide is diluted in the overlying water before contacting the submersed foliage and rapidly photodegrades in water (Pest Management Regulatory Agency, 2020). More, even if submersed aquatic vegetation damage occurred from intended overtreatment into water, detection in the post-treatment imagery would not be evident in the open water. However, when the vegetation bordering *P. australis* patches was other emergent plant species, non-target damage more closely resembled intended overtreatment. This neighboring non-target emergent vegetation damage we attribute mainly to the RPAS's swath width and the intended overtreatment along the edges of *P. australis* stands necessary to capture all the *P. australis* ramets. Importantly, when non-target vegetation was interspersed within the *P. australis* patch, non-target plant damage could not be characterized independently from *P. australis* death in the

overlay analysis. Non-selective herbicide spray applications will damage any vegetation, including non-target plants growing within a *P. australis* patch.

Our approach to deriving the intended treatment areas involved buffering the RPAS's flight path with the 3 m estimated swath width to calculate the footprint to which herbicide was applied by the RPAS while it was actively spraying (Figure 4-2), so it is important to note that these treated area values are an estimate. A study by Rasmussen et al. (2019) found that log files for ground-based patch spraying consistently overestimated the sprayed area, suggesting that where the sprayer thought it had sprayed and evidence of spray deposition in the field were not aligned. Contrary to the findings of Rasmussen et al. (2019), there was strong agreement between the intended overtreatment areas and the non-target plant damage areas in our study, with the exception being when *P. australis* was surrounded by open water. Based on where we think herbicide was applied outside of the *P. australis* area from the extracted flight paths and pre-treatment imagery, there was clear evidence of emergent vegetation mortality in the post-treatment imagery. This suggests that in intended overtreatment areas, herbicide application occurred at a high enough concentration to effectively damage any emergent vegetation in that area. Differences in intended overtreatment and non-target areas can be attributed to several of our target *P. australis* treatment areas being surrounded by open water, where submersed aquatic vegetation are not likely to be adversely impacted by Habitat® Aqua application and visual evidence of plant mortality in the open water of the post-treatment imagery is not detectable.

While pesticide drift from RPAS-based applications has been extensively studied with indicators (e.g., Chen et al., 2020; Gibbs et al., 2021; Grant et al., 2022), the ecological effects of that drift and the quantity of drift likely to provoke a toxic response in surrounding vegetation is comparatively understudied. In wetlands, the presence of emergent native vegetation surrounding

P. australis provides a distinct footprint of the effects of herbicide drift outside of the application area. We observed a range of drift, both relative to the area of *P. australis* targeted for treatment (4 – 34%), and the total area that was sprayed by the RPAS (< 2 – 16%). Differences in drift rates across intended treatment area suggest that herbicide drift was variable, even when the automated flight parameters were held constant. The addition of semi-assisted flights completed along the perimeter of each patch after the initial automated flight was a unique factor for each intended treatment area and may have contributed to the variation in drift rates. As the semi-assisted flight only targeted *P. australis* ramets along the edge that may not have had adequate spray coverage from the initial passes, it is possible that the boundaries of the application area were overestimated.

Spongy Lake exhibited the greatest drift (15 – 34% of *P. australis* area and 6 – 16% of treatment area), despite having relatively low perimeter-area ratios (approximately 0.08 at Spongy Lake) compared to the other sites (> 0.10 at Baie du Doré and Rondeau Provincial Park). Environmental wind was also low (1.1 kph at the edge of the lake) during this treatment due to the lake being sheltered by trees. We do not think this was the result of temperature inversions, despite the low wind speed (Government of Canada, 2020) because wind velocity measurements in the farm field surrounding the depression in which the kettle lake is situated were roughly 30 kph before entering the lake (Janice Gilbert, pers. comm.). A possible reason greater drift was observed at Spongy Lake could be the sparseness of the *P. australis* stands. While no on-the-ground measures of stem density were completed at Spongy Lake, visual examination of high-resolution aerial imagery of the *P. australis* stands compared to Rondeau Provincial Park and Baie du Doré suggests a lower stem density. A lower density of foliage for the herbicide droplets

to intercept may leave more droplets susceptible to being pushed through the canopy or drifting laterally outside the application area (Kim et al., 2011).

In contrast, drift at Baie du Doré and Rondeau Provincial Park were lower (4 – 14% of targeted *P. australis* area and 2 – 9% of treatment area), with no clear relationship between drift and patch size or perimeter-area ratios. Lower drift rates at Rondeau Provincial Park were likely related to the greater undertreatment observed (13 – 17%). As *P. australis* grew beneath a tree canopy that bounded one side of the treatment patches in the swamp slough, spraying to the edge of the patch was not always possible, reducing the opportunity for herbicide drift to non-target vegetation along that boundary. At Baie du Doré, the open marsh lacked obstacles like trees where *P. australis* existed. However, the presence of open water around a large proportion of the patches meant that herbicide drift into the surrounding water could not be detected in the post-treatment remote-sensing classification.

Drift percentage by perimeter was more consistent across all intended treatment areas than drift rate by area. Generally, drift occurred along approximately 25% of the intended treatment area's perimeter. This suggests that herbicide applied by the RPAS tended to drift in one direction or to one side of an intended treatment area. In most intended treatment areas, a larger drift footprint was evident on one side, even if smaller drift occurred along multiple sides (Appendix B). This larger footprint may have been caused by the final pass from the semi-assisted flights, potentially in combination with a wind gust in the drift direction. The relative consistency in drift proportion by perimeter of the intended treatment area suggests it may be better at quantifying the opportunity for drift to occur compared to patch size or perimeter-area ratio. This contrasts with helicopter spraying, where *P. australis* patch area may play a larger role in herbicide drift due to larger patches requiring fewer passes from a helicopter compared to

an RPAS. However, the larger swath widths and faster flight speeds from helicopters can result in more herbicide drifting outside the target area, especially when the patches are smaller than the swath width (Teske et al., 1998). For example, at Baie du Doré, the smaller treatment area required approximately seven passes by the RPAS to treat the full area (Fig. 4-1), covering the entire 25 m of width, including the semi-assisted flight. A helicopter with a 25 – 30 m swath, would need only one pass for the same patch (Yao et al., 2021). Additionally, the time between turning the nozzles on and off when flying a helicopter can lead to additional drift perpendicular to the swath (Robinson et al., 2000) due to greater flight speeds. Therefore, when patches are larger than the RPAS's swath width (~3 m), drift is reduced to the passes along the patch edges, and interior drift is negligible as it is more likely to land within the treatment area.

The maximum distance of drift observed from all our intended treatment areas was approximately 20 m, with the next highest value only near 9 m. Our average distance aligns with the drift distance of 8 – 10 m reported by Chen et al. (2020) when spraying pesticides with an eight-rotor RPAS using a medium droplet size. While the range of maximum drift distance was large (< 3 m – 20 m), the average value was roughly 8 m, with six out of seven intended treatment areas having drift less than 10 m from the application area. This suggests that drift to non-target vegetation is unlikely to occur beyond 10 m from the application area in most applications, provided similar flight parameters are used. Even when considering potential damage to non-target plants at a 20 m distance, drift distance from the RPAS is nearly 78% less than the distance of vegetation damage observed with helicopter spraying of glyphosate (90 m; Hogg, 2018) and 96% less than the distance of damage to non-target vegetation that could be observed during a helicopter-based application of Habitat® Aqua to *P. australis* (450 m; BASF Canada Inc., n.d.). In fact, even ground-based applications suggest a buffer zone of at least 30 m

from non-target vegetation when using Habitat® Aqua for *P. australis* suppression (BASF Canada Inc., n.d.). While there are currently no mandatory buffer zones for Habitat® Aqua use in wetlands, regardless of application type, we suggest that RPAS-based herbicide applications maintain a minimum buffer of 20 m from sensitive species as a precautionary measure. This value closely aligns with the recommended buffer zone of 15 m – 25 m for RPAS-based pesticide application of row crops reported by Gibbs et al. (2021). Additionally, the use of a semi-assisted flight is valuable to ensure spray quality along the edges of *P. australis*; however, due to the increased risk of drift along the perimeter, consideration should be given to reducing flight height (Huang & Thomson, 2011) and/or speed (Hunter et al., 2019) during these perimeter applications.

The on-target damage was highest at Baie du Doré (nearly 100%) and lowest at Rondeau Provincial Park (85%). Despite the variation in on-target damage derived from the post-treatment imagery, the on-the-ground transects measured a similar number of live *P. australis* stems at each site (six at Baie du Doré and nine at Rondeau Provincial Park). The suppression efficacy quantified at the site-level for Baie du Doré and Rondeau Provincial Park was roughly a 98% reduction in live stems. Differences observed in the on-target damage sprayed and the number of live *P. australis* stems may be due to streaking between flight passes caused by wind gusts or insufficient spray quality at the edges, where the semi-assisted flight was unable to correct. Both the transect and the quadrat method (surveyed 1 m² plots, Chapter 1.0) of surveying agree that *P. australis* suppression efficacy was very high (> 95%), and as high or higher than reported in previous studies (e.g., Mozdzer et al., 2008; Whyte et al., 2009; Knezevic et al., 2013). The minor difference in estimates of suppression efficacy (approximately 98% from the transects and

> 99% from the quadrats) corroborates that the RPAS-based herbicide applications were highly successful at reducing *P. australis* live stem density after a single treatment.

A significant trade-off between undertreatment and intended overtreatment areas was found in our RPAS-based herbicide applications to *P. australis*. When many smaller patches are too close together to justify spraying each individually, some overtreatment may be unavoidable. When interspersed non-target species are also non-native species (e.g., *Typha x glauca*, *Hydrocharis morsus-ranae*, etc.), the vegetation damage from intended overtreatment may be justified for more complete suppression of *P. australis* given its high capacity to re-establish if it survives the initial application (Elsey-Quirk & Leck, 2021). However, where more sensitive and desirable native plant species exist near *P. australis* patches, individual patch spraying should be considered. Currently, such high precision for herbicide spray applications is only possible with backpack spraying. RPAS application holds the potential to enable spot-spraying of small *P. australis* clusters as it can hover directly over canopies, but research is needed to test the accuracy of spot-spraying. Where *P. australis* patches are uniform and dense (> 20 live stems/m²), RPAS-based herbicide application can effectively target the patch with a fraction of the drift observed from helicopter spraying, achieving high (> 95%) suppression efficacy and representing a new balance point in the trade-off between overtreatment and undertreatment.

4.5 Conclusions

RPAS-based herbicide application to *P. australis* patches at four wetlands achieved an average of 91% on-target accuracy. Low undertreatment rates presented a trade-off with considerable overtreatment, attributed partly to the complex shape of *P. australis* patches and the intermixing with non-target plants along patch margins. Some intended overtreatment is

unavoidable to mitigate the risk of *P. australis* survival and regrowth, but some reduction in intended overtreatment could have been achieved through more accurate differentiation of *P. australis* and *Typha x glauca*. Achieving accurate herbicide application while minimizing non-target impacts represents an area where RPAS-based herbicide application could be particularly valuable, especially in treating patches of *P. australis* surrounded by desirable native emergent plants. Herbicide drift from the RPAS applications was found up to 20 m away from the application area but was 78% less than observed vegetation damage from helicopter spraying of glyphosate and 96% less than the possible distance vegetation damage could be observed when spraying Habitat® Aqua via helicopters. Drift was possibly exacerbated by semi-assisted flights that were implemented to ensure thorough treatment of *P. australis* patch edges. We recommend lower flight heights and/or reduced flight speeds be used during these semi-assisted patch-perimeter flights. Ultimately, effective *P. australis* management requires balancing control efforts with conservation goals. Our results highlight the promising capabilities of RPAS-based herbicide application for uniform and dense *P. australis* patches. However, the unique distribution of *P. australis* as it grows unconfined in wetlands poses challenges for precise herbicide application, even with RPAS technology. Future research should focus on refining application accuracy (e.g., through spot-spraying) to reduce intended overtreatment and collateral damage to non-target species, especially where small clusters of *P. australis* are irregularly distributed among other, desirable vegetation.

5.0 Conclusions and recommendations

5.1 Thesis overview

The persistence of *Phragmites australis* subsp. *australis* invasions (hereafter *P. australis*) in many wetlands can be attributed to limitations in available control methods. The rapid spread and dominance of *P. australis* threatens sensitive species by significantly altering habitats. If left uncontrolled, these invasions lead to a decrease in overall biodiversity and habitat degradation (Zedler & Kercher, 2004). *Phragmites australis* patches that are small (< 0.01 ha), interspersed with other vegetation, and/or inaccessible are often overlooked during wetland management. This is due to the costs associated with controlling these invasions, which are often outweighed by the effort and disturbance required to access the sites and complete treatment, or the environmental impact of broad-scale herbicide application. However, the likelihood of eradicating *P. australis* is greatest when targeting small patches and neglecting to control invasions at this stage allows *P. australis* to spread to patch sizes that are unlikely to be eradicated by management efforts (Quirion et al., 2018).

To improve *P. australis* control efforts, a spray-equipped remotely piloted aircraft systems (RPASs) applied Habitat® Aqua to invasions of *P. australis* at four pilot sites, representing wetland environments where traditional herbicide application has been constrained by access, landscape features, and the presence of sensitive species. An additional site was integrated in the following year to assess the herbicide droplet deposition of the RPAS-application on a *P. australis* canopy.

Our goals in this thesis were to 1) to evaluate the *P. australis* suppression efficacy of RPAS-based herbicide application in wetlands, 2) to quantify vertical and lateral herbicide droplet movement when applied by an RPAS to *P. australis* with medium and coarse droplet sizes, and 3) to assess the application accuracy and post-treatment effects of RPAS-based herbicide applications to *P. australis*.

5.2 Thesis summary

In the first data chapter, we evaluated the suppression efficacy of RPAS-based herbicide application and characterized the extent of native plant recovery one year after herbicide treatment. We conducted a spatially replicated vegetation sampling design across 36 plots at Baie du Doré and Rondeau Provincial Park Great Lakes coastal wetlands. We determined that RPAS-based herbicide application effectively suppressed *P. australis* by reducing both live *P. australis* stem density and canopy height in the established plots. However, there were concurrent reductions in species richness, evenness, and diversity in the first year after the herbicide application. While the mean coefficient of conservatism in the herbicide treated plots did not change, dominance of the native species *Impatiens capensis* in nearly all plots at Baie du Doré showed the potential for native plant recovery where intact seedbanks are present. Conversely, secondary invasion by *Hydrocharis morsus-ranae* was observed after the first year of herbicide treatment at Rondeau Provincial Park, highlighting the complex ecological responses to *P. australis* suppression in wetlands. The widespread presence of *H. morsus-ranae* in pre-treatment surveys of the targeted *P. australis* patches in Rondeau suggests that pre-treatment surveys could help predict where secondary invasions present a greater risk.

In the second data chapter, we used water-sensitive papers as indicators to quantify herbicide droplet deposition through the vertical plane of a *P. australis* canopy and the lateral spread of droplet drift away from the application area. We also compared these depositions between medium and coarse droplet sizes. We determined that percent area coverage was not different among heights within the *P. australis* canopy for either droplet size, nor did droplet size have a significant effect on the vertical penetration. The exception to this was the volume median diameter (VMD) of the coarse droplets was larger than the medium droplets at the lowest canopy height, suggesting greater ground-loss potential is possible with a larger droplet size due to the combined gravitational and downward propulsion forces from the RPAS rotors. Lateral droplet drift occurred at least 8 m away from the application area with medium droplet presence decaying at a faster rate than the coarse droplets, even under relatively low wind conditions. This contradicted the idea that smaller droplets have higher drift potential due to their lower mass and higher susceptibility to wind displacement, suggesting RPAS downwash forces may have a greater influence on the movement of herbicide droplets compared to environmental wind speeds, which are required to be low during application by the Pest Management Regulatory Agency label instructions.

In the third data chapter, we used remotely-sensed images of four wetlands, before and after RPAS-based herbicide application to assess the application accuracy and post-treatment effects on vegetation. We determined that while RPAS-based application was able to accurately target *P. australis* roughly 90% of the time, there was a notable trade-off between the area of wetland subject to undertreatment and intended overtreatment. The non-target effects observed after herbicide treatment corresponded well with the intended overtreatment area derived before herbicide treatment, indicating that our method for estimating the intended treatment area by

buffering the RPAS flight path with the swath width was accurate. Herbicide drift resulted in vegetation death up to 20 m away from the application area and the drift footprint could be further reduced by lowering flight height and speed around the *P. australis* patch edge. We conclude that RPAS-based herbicide application offers a viable alternative to helicopter applications that minimizes the drift footprint outside of the intended application area.

5.3 Research implications

Our research contributes to the existing body of knowledge on spray-equipped RPAS for precision pesticide application by providing new insights into their capacity to accurately target and effectively suppress invasive *P. australis* in wetland environments. Specifically, we found that RPAS-based herbicide applications achieved equal, or higher *P. australis* suppression efficacy compared to currently-available herbicide application methods with substantially less off-target damage from herbicide drift than is expected from helicopter applications. Our studies provide a comprehensive overview of the plot- (Chapter 2.0), patch- (Chapter 3.0), site-level (Chapter 4.0) impacts of RPAS-based herbicide application to *P. australis*. These results will ultimately contribute to the evaluation of RPAS technology by the Pest Management and Regulatory Agency by informing the efficacy and safety of this application method for *P. australis* suppression across Canada.

Our results are valuable for assessing RPAS technology as a new method for herbicide application to *P. australis* in sensitive wetlands. This method combines higher application precision, analogous to backpack spraying, with the advantages of aerial application for inaccessible *P. australis* invasions. The high precision provided by backpack spraying is the only foliar spray method that can treat small or clustered patches of *P. australis*. However, where

these invasions exist in inaccessible areas, wetland managers are unable to effectively suppress *P. australis* without considerable collateral damage from helicopter spraying. Approving use of this technology would enhance wetland manager's capacity to control *P. australis*, particularly where available methods are limited by patch size, configuration, and/or access. RPAS technology also shows promise for follow-up treatments, with the capacity to spot-spray remnant or sparse ramets along patch boundaries without reapplying large quantities of herbicides across previously treated areas. Such applications present the next frontier for this research.

Currently-available methods for *P. australis* control range in their relative precision (e.g., individual ramet spading being the most precise and herbicide application by helicopter being the least). While control method selection will depend on the size and density of *P. australis* stands, accessibility of the site to be treated, and presence of sensitive species, the highest precision of control cannot always be employed based on the extensive current distribution of *P. australis* invasions and the rate at which it can spread in wetlands (Catling & Mitrow, 2011). Use of herbicides is often necessary for effective initial *P. australis* suppression (Hazelton et al., 2014), however, overtreatment from helicopter spraying represents an unbalanced trade-off in wetland restoration. One of the reasons there are currently constraints in our ability to control many inaccessible *P. australis* invasions is because RPAS technology is not yet approved for aerial herbicide applications to *P. australis*. Approving this application method would reduce labour requirements and off-target impacts to surrounding vegetation and provide an additional tool in wetland restoration, enabling wetland managers to better balance the trade-off between overtreatment and undertreatment. Specifically, we recommend adoption of RPAS-based herbicide application for controlling small, clustered *P. australis* patches in wetlands that previously persisted due to constraints in available herbicide application methods.

5.4 Future work and recommendations

This thesis represents an integrated assessment of RPAS technology for precision herbicide applications to *P. australis* in wetland environments. Our research encompassed the first-ever RPAS-based herbicide application to *P. australis* in Canada, unveiling the initial suppression efficacy, herbicide droplet deposition, and site-level treatment effects. Importantly, our findings have instigated further research opportunities to continue refinement of this novel herbicide application method.

The first year of post-treatment vegetation monitoring represents a starting point for continuous monitoring of vegetation community changes. However, long-term monitoring of RPAS-based herbicide treated plots is recommended to ensure re-establishment of *P. australis* does not occur and to determine if the plant community follows a recovery trajectory and timeline similar to those reported in the literature (Zimmerman et al., 2018; Bonello & Judd, 2020). Continued monitoring will also provide insight on the secondary invasion of *Hydrocharis morsus-ranae* at Rondeau Provincial Park and the establishment of *Impatiens capensis* at Baie du Doré or identify if colonization by other species occurs in the open niche left after herbicide treatment. The dynamic water levels in the Great Lakes may influence species establishment after herbicide treatment in Great Lakes coastal wetlands, making long-term monitoring of these conditions crucial for understanding the factors affecting their plant community recovery.

Our assessment of RPAS-based herbicide applications involved the use of two quadcopters (Hyllo Inc. equipped with eight AIXR110015 spray nozzles in Chapters 2.0 and 4.0; DJI equipped with four TT110015 nozzles in Chapter 3.0). As our experiments occurred in different years and with different RPAS models, cohesion among the suppression efficacy, post-

treatment effects, and in-field application data collected could not be synthesized. We recommend that further studies on RPAS-based herbicide applications use multiple indicator types (e.g., fluorescent or colorimetric tracer) to overcome the limitations from a single indicator (e.g., those reported for water-sensitive papers). Indicators should be placed in areas where field sampling, remote sensing, or other techniques are being used to evaluate herbicide treatment effects. This approach will allow the quantification of the amount of herbicide applied necessary to achieve a given sufficient suppression efficacy, along with the minimum effective dose of herbicide able to damage non-target vegetation.

A critique in the literature pertaining to RPAS technology is the lack of consistency in parameters across or within studies. These parameters can be diverse, with countless configurations of nozzles, numbers of rotors, types of nozzles, flight heights, flight speeds, and sizes of RPAS's that could be employed for herbicide application to *P. australis*. Future studies should focus on optimizing parameters to enhance foliar coverage, while reducing ground-losses and lateral drift. A specific focus should be directed to how much to lower flight heights and speeds along *P. australis* patch edges to minimize product drift that was observed in our study (Chapters 3.0 and 4.0).

We assessed the performance of RPAS-based herbicide applications with nozzles placed directly below the rotors and all actively spraying to create a swath over the targeted *P. australis* patches. However, by employing only one or a few nozzles at a time, RPAS technology holds the potential as a high-precision spot-sprayer for use in follow-up treatments. Studies are needed on how accurately an RPAS could target and spray individual or small groups of *P. australis* ramets. Challenges in spot-spraying *P. australis* may include being able to identify remnant ramets if secondary control efforts do not remove standing dead biomass and understanding how

rotor propulsion affects the ability for the RPAS to target *P. australis* at this fine-grain level of control. Spot-spraying herbicide with an RPAS could improve follow-up control efforts by reducing environmental risk and labour requirements when remnant ramets are dispersed. Additionally, RPAS technology would address the need for follow-up treatments in inaccessible sites that may be overlooked after initial broad-scale herbicide treatment.

These studies were the first to evaluate RPAS technology to apply herbicides to invasive *P. australis* in wetlands. Our research fills specific gaps in the literature regarding the suppression efficacy of RPAS-based herbicide application, its performance on a *P. australis* canopy, and the resulting footprint of herbicide treatment effects. Our results provide valuable information for *P. australis* management, demonstrating how this tool can optimize suppression of the target species while minimizing damage to the surrounding environment. This ultimately addresses concerns associated with excessive overtreatment from helicopters, currently the only permitted aerial application method for use on *P. australis* in wetlands.

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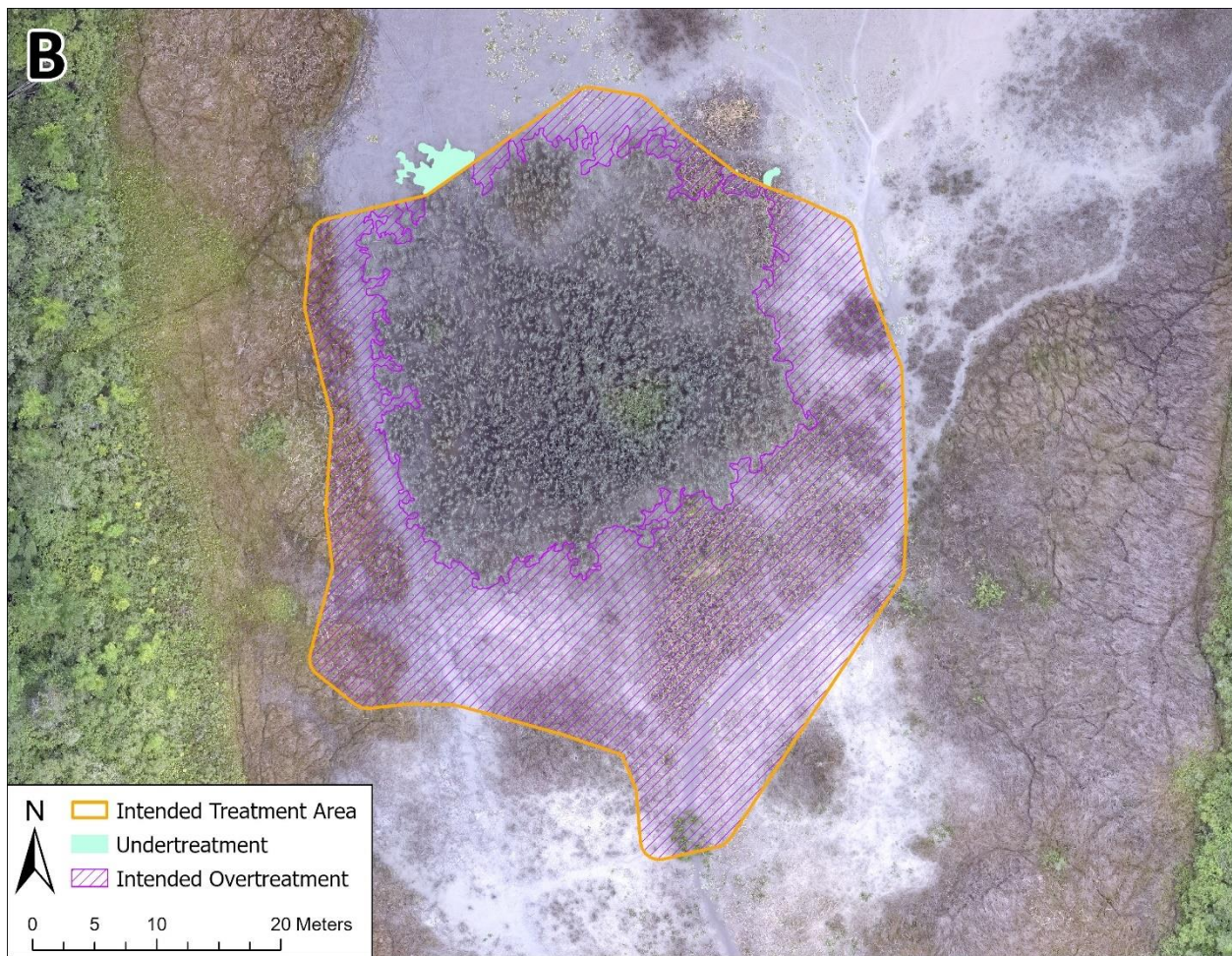
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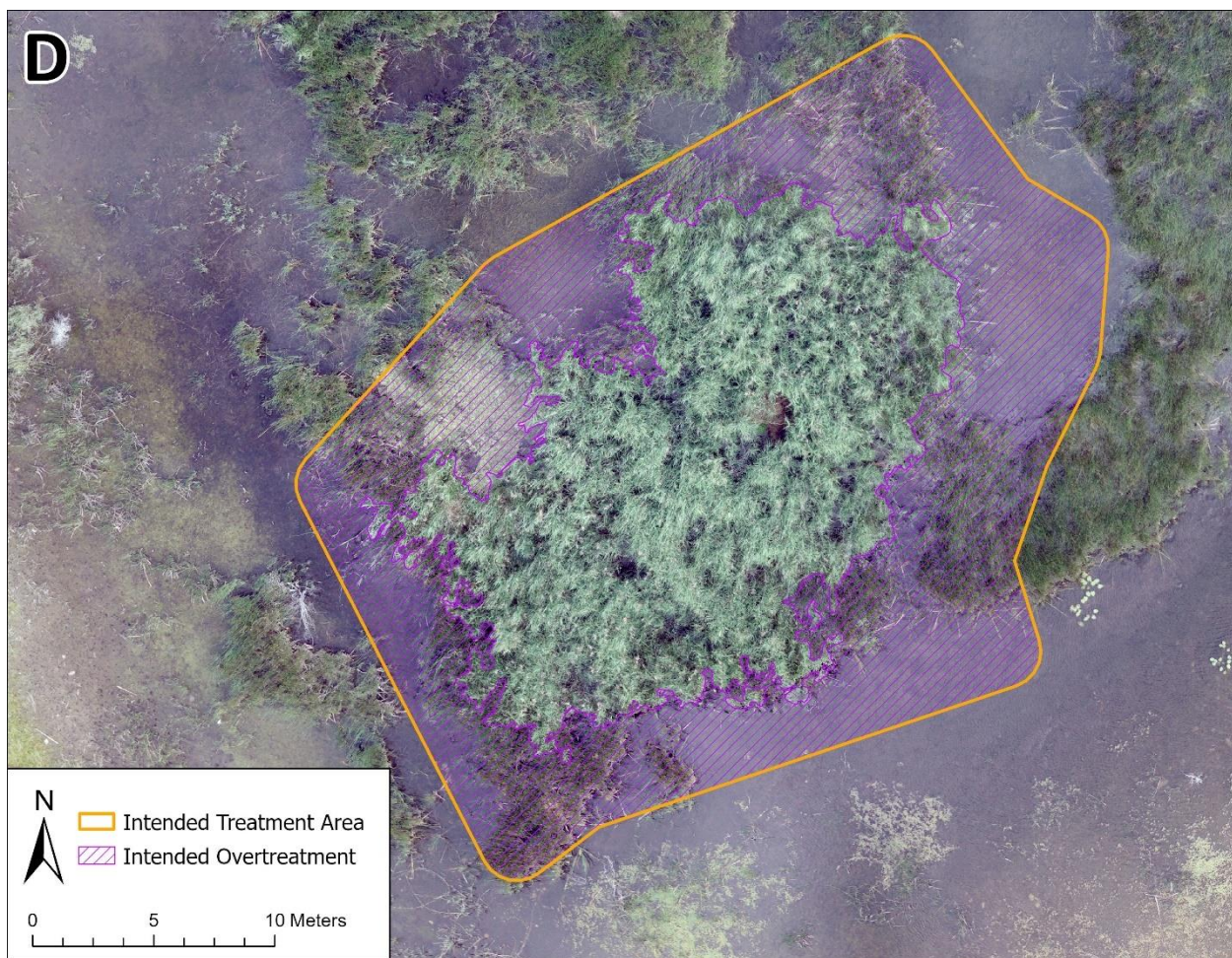
Appendices

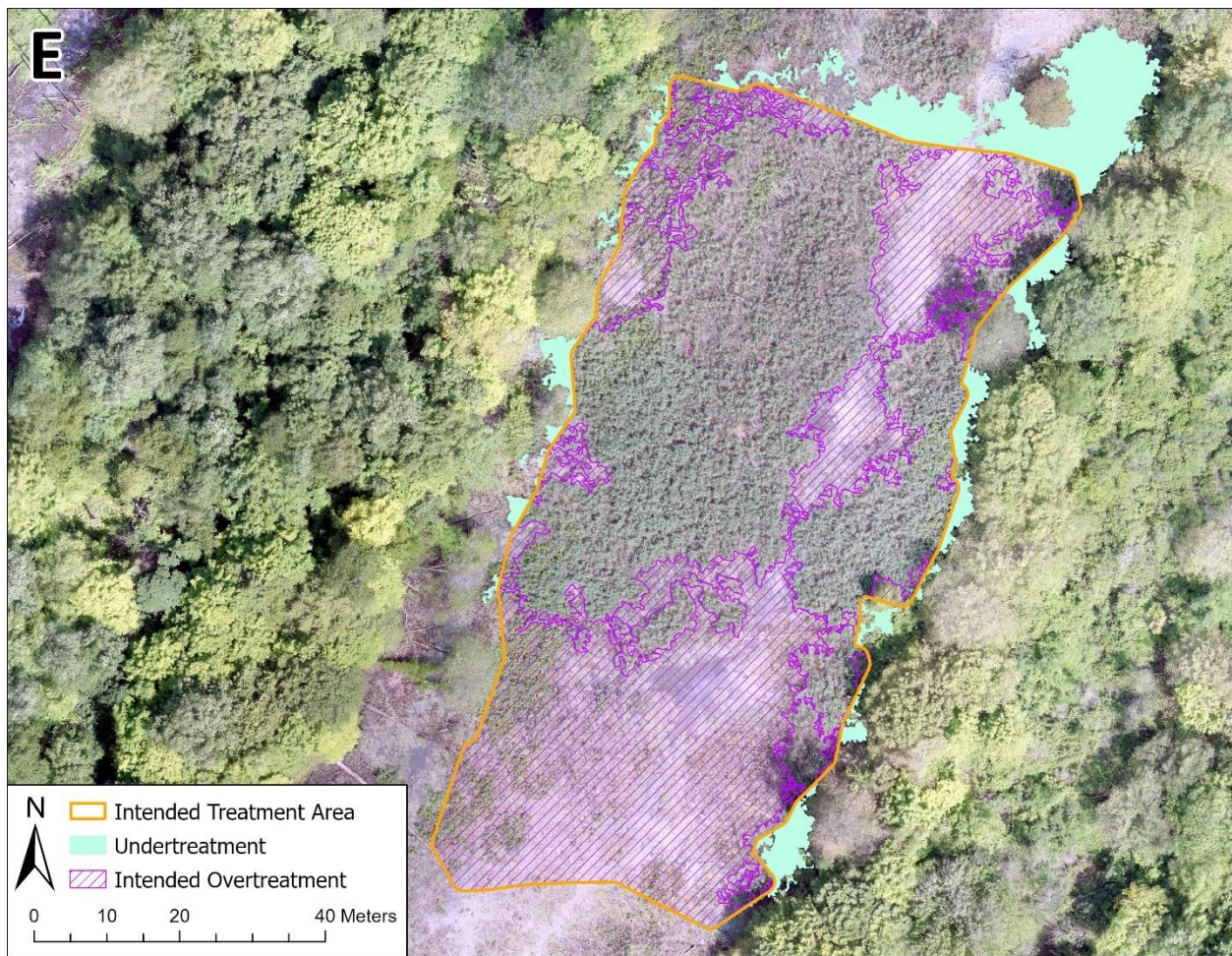
Appendix A. Maps of undertreatment and intended overtreatment associated with the intended treatment areas by an RPAS at Spongy Lake, Baie du Doré, Rondeau Provincial Park, and Wood Drive wetlands. Maps depict treatment polygon ID's SL-North (A), SL-South (B), BDD-North (C), BDD-South (D), RPP-North (E), RPP-Centre (F), RPP-South (G), WD-North (H), WD-Middle (I), and WD-South (J). Basemap imagery was collected by Ontario Power Generation's Advanced Inspection and Maintenance team in 2022.

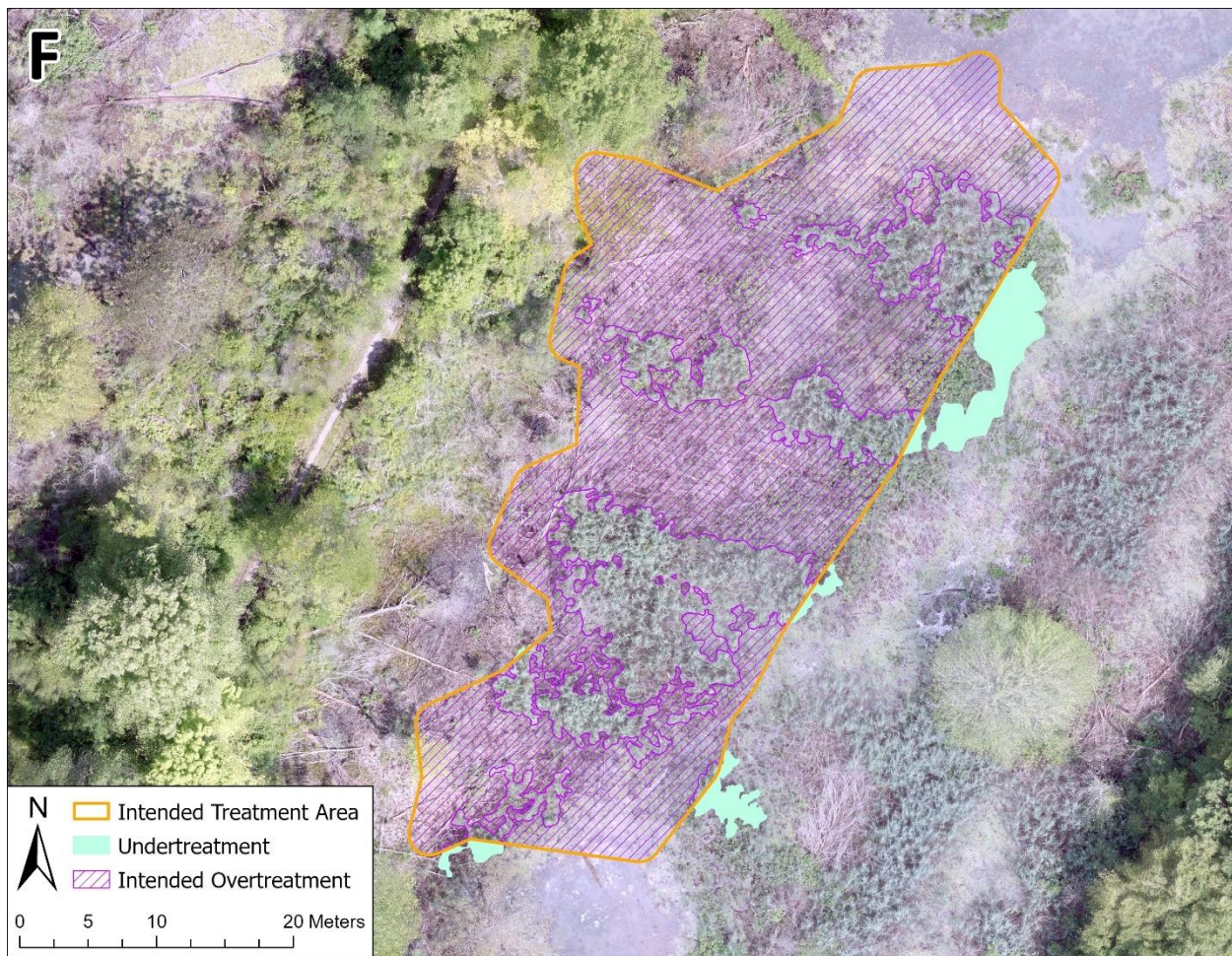


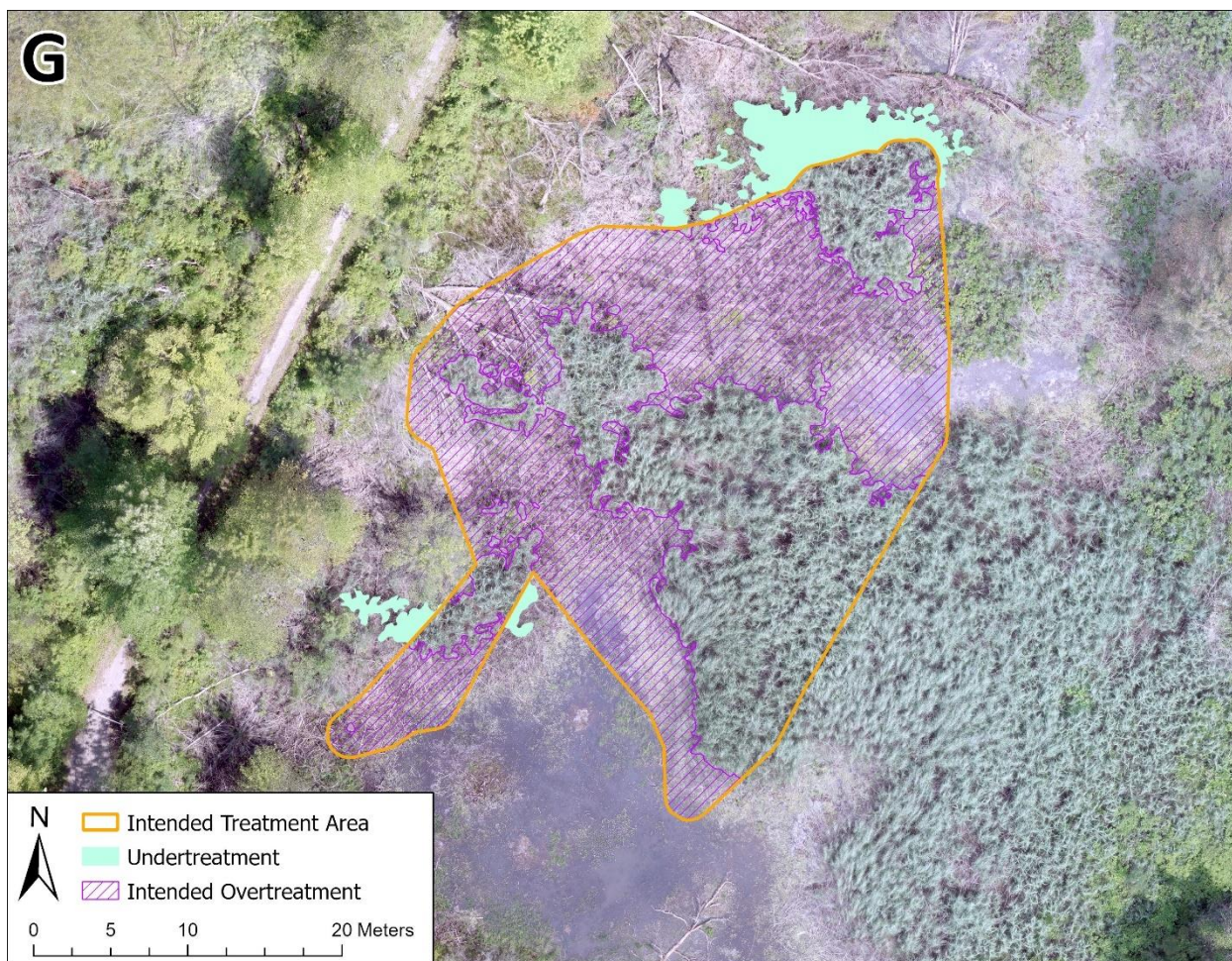


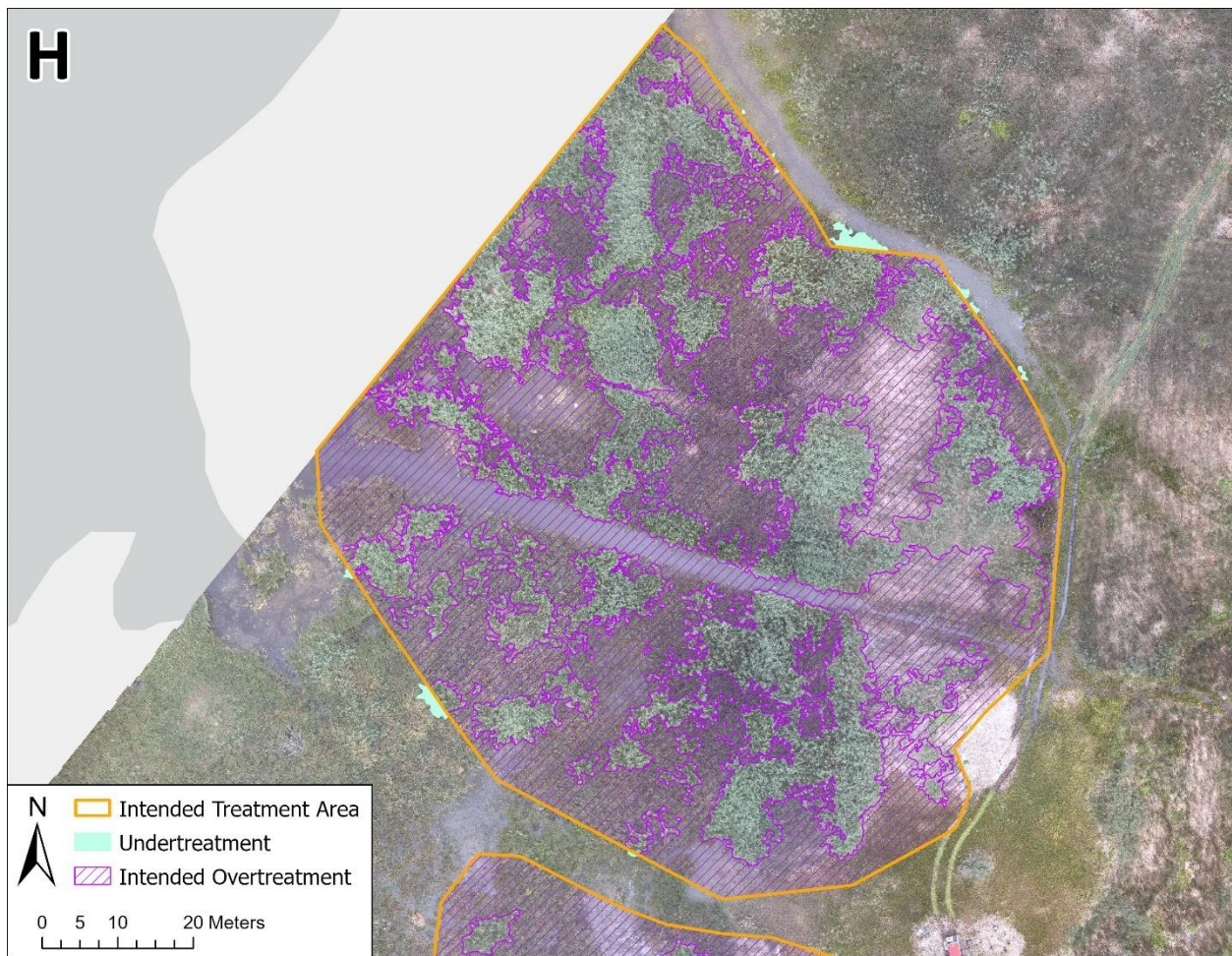


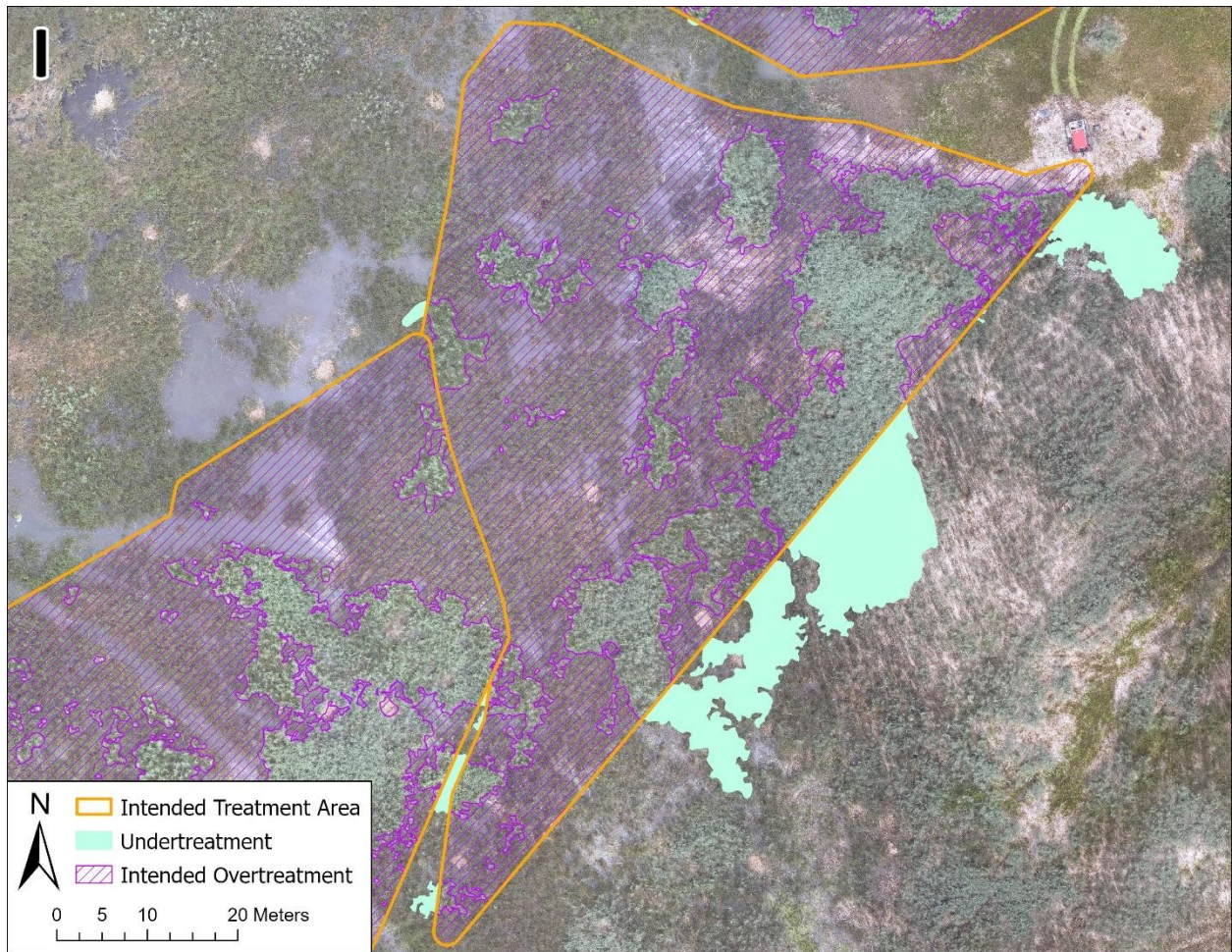


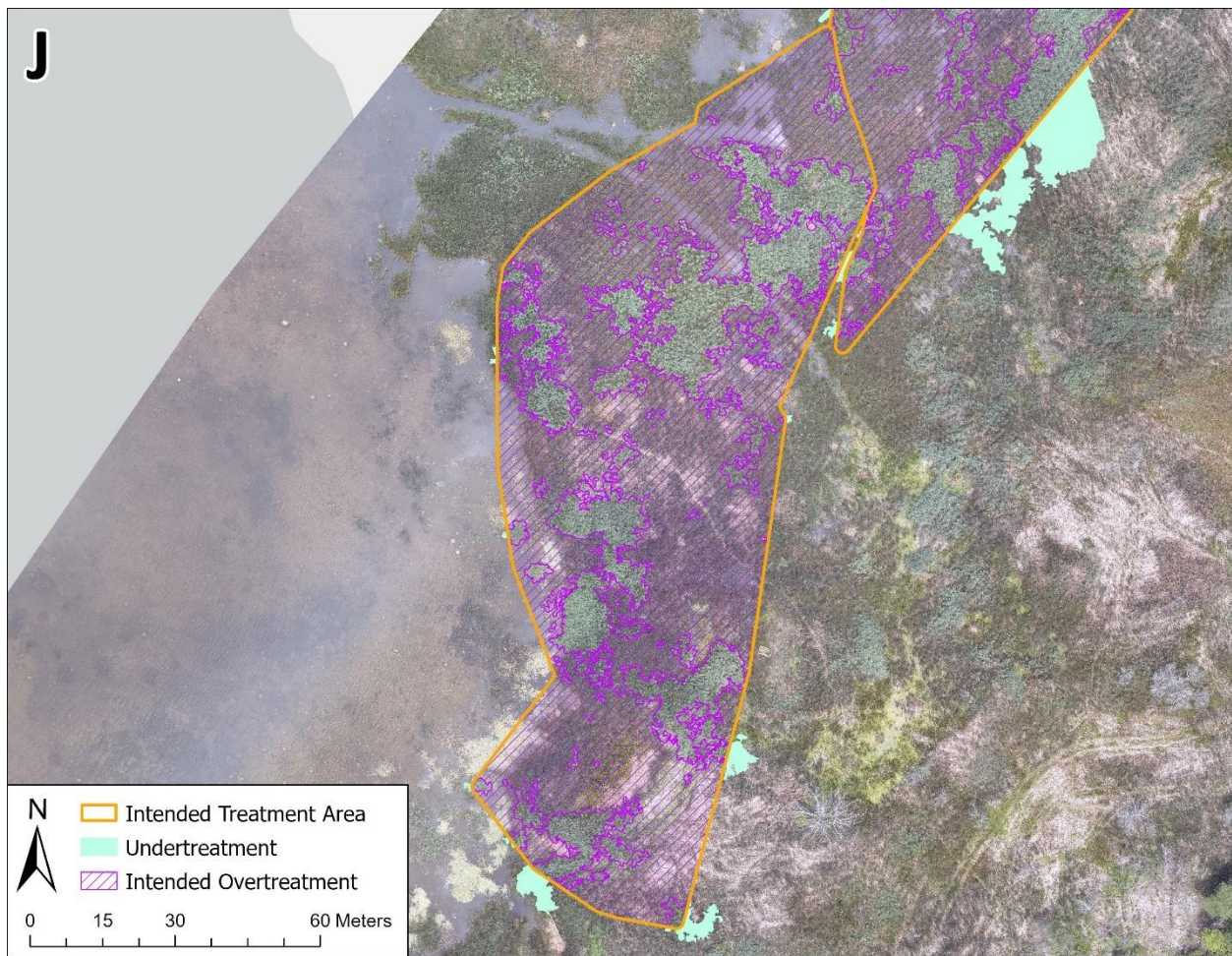






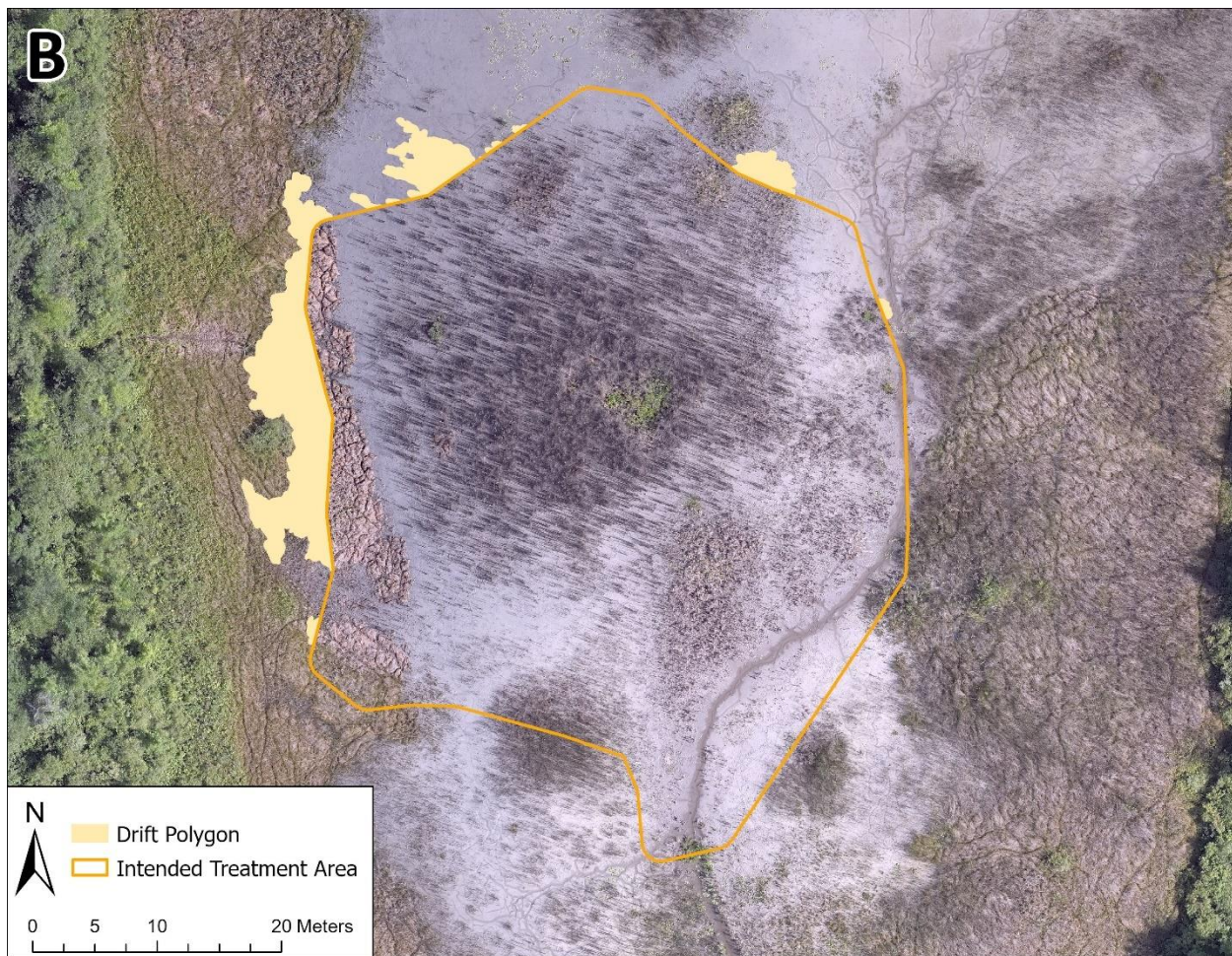


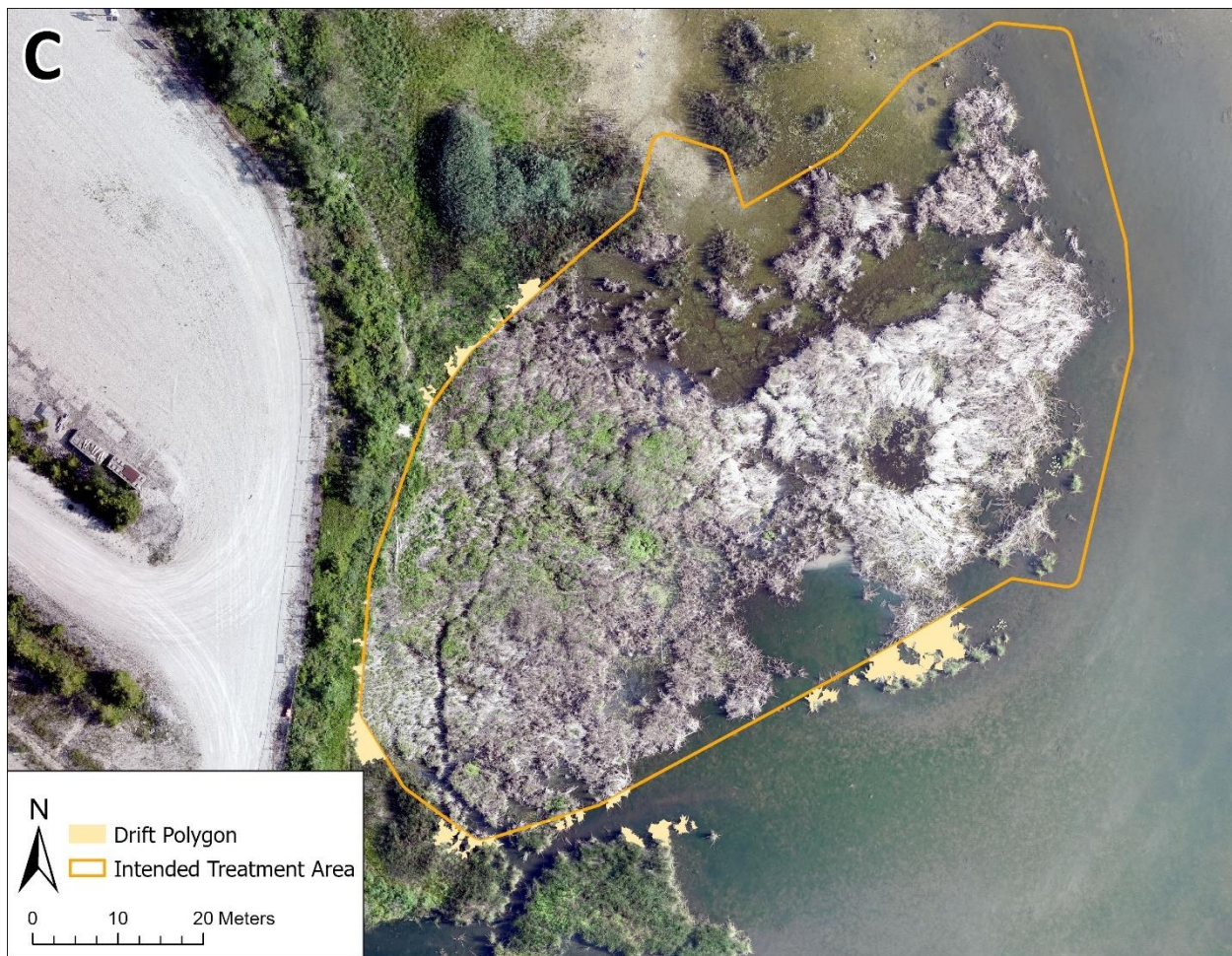




Appendix B. Maps of RPAS-applied herbicide drift footprint in relation to the intended treatment areas at Spongy Lake, Baie du Doré, and Rondeau Provincial Park wetlands. Maps depict treatment polygon ID's SL-North (A), SL-South (B), BDD-North (C), BDD-South (D), RPP-North (E), RPP-Centre (F), and RPP-South (G). Basemap imagery was collected by Ontario Power Generation's Advanced Inspection and Maintenance team in 2023.















Appendix C. Raw data associated with the three data chapters can be found at:
<https://doi.org/10.6084/m9.figshare.c.7348129>.