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AQUATIC PLANT CONTROL RESEARCH PROGRAM

Unmanned Aircraft Systems and Tracer Dyes

Potential for Monitoring Herbicide Spray Distribution

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PURPOSE: Chemical control of nuisance aquatic vegetation has long been the most widely utilized management tool due to its high level of efficacy, limited environmental impacts, and relatively low cost. However, unprecise application of herbicides can lead to uncontrolled invasive plants and unintended management costs. Therefore, precision herbicide delivery techniques are being developed to improve invasive plant control and minimize impacts to non-target plants. These technological advancements have the potential to enhance aquatic ecosystem protection from invasive species while reducing associated management costs. Despite the benefits of using registered herbicides for aquatic plant control in efforts to restore aquatic habitats, their use is often misunderstood and opposed by public stakeholders. This can lead to significant challenges related to chemical control of nuisance aquatic vegetation. Thus, US Army Corps of Engineers (USACE) Districts seek improved methods to monitor and quantify the distribution (i.e., amount of herbicide retained on plant foliage compared to those deposited into the water column) of herbicides applied in aquatic systems. Monitoring herbicide movement in aquatic systems can be tedious and costly using standard analytical methods. However, since the inert fluorescent tracer dye Rhodamine WT (RWT) closely mimics product movement in the aquatic environment it has been used as a cost-effective surrogate for herbicides tracing.

The use of RWT (or other inert tracer dyes) can be an efficient way to quantify herbicide retention and deposition following foliar treatments. However, the collection of operational spray deposition data in large populations of invasive floating and emergent plant stands is labor intensive and costly. One proposed solution is the use of remote sensing methods as an alternative to traditional *in situ* samples. Specifically, using unmanned aircraft systems (UAS) in conjunction with RWT could provide more efficient monitoring and quantification of herbicide spray distribution and in-water concentrations when using RWT in combination with herbicides. A better understanding of UAS capabilities and limitations is key as this technology is being explored for improved and integrated management of aquatic plants in the U.S. This technical note (TN) provides a review of literature to assess the state of knowledge and technologies that can assist USACE Districts and partners with tracking herbicide movement (using RWT as a surrogate or additive), which could improve operational monitoring, thus reducing the level of uncertainty related to chemical applications and non-target impacts, and thus improve management in aquatic systems.

INTRODUCTION: Pesticides are utilized in the US to control unwanted plants, insects, or pathogens, and are typically applied as a liquid spray targeting the foliage, insects, or pathogens directly with the goal of achieving complete coverage (Dorr et al. 2015). A successful treatment applied to the foliage remains on the surface of the target species and the solution/droplets are not lost as spray drift or deposited on the ground (or water) (Dorr et al. 2015). Historically, herbicides are one of the most cost-effective and resource-efficient tools to manage problematic aquatic plants

(Mudge et al. 2021). For each herbicide active ingredient, the US Environmental Protection Agency (USEPA) conducts ecotoxicological risk assessments for non-target organisms to grant or deny registration for aquatic use (USEPA 2019). However, data generated from USEPA aquatic metabolism studies do not consider any herbicide spray solutions being intercepted by plants growing at or above the water surface (i.e., floating, emergent, or creeping plants), neither are water depths accounted for, which can influence final herbicide concentrations (Mudge et al. 2021). Although maximizing herbicide retention onto the foliage of target species is a management goal for improved efficacy, product can enter the water column due to a lack of complete plant coverage (required to intercept spray solution), via abiotic factors (i.e., increased carrier volume and spray angle), or off-target movement from leaves and stems. Quantifying the amount of herbicide entering the water column would provide a more precise assessment of product not retained by the plant and could shape future toxicology risk assessments for non-target species.

Research is needed to better address herbicide spray patterns and deposition into the water column while conducting emergent and floating aquatic vegetation treatments. Study findings stand to improve precision of foliar herbicide applications and optimize use patterns as well as determine the amount of product reaching the water column under various application processes and conditions. A thorough literature search was conducted and found limited publications related to foliar herbicide deposition in aquatic sites where floating/emergent/creeping plants were managed. In one study, Anderson et al. (1983) reported 10% to 20% of 2,4-D and diquat applied to water hyacinth [*Eichhornia crassipes* (Mart.) Solms] in California's Sacramento Delta, was captured on floating petri dishes placed under plant canopies. Similarly, an in-water concentration of 0.46 µg L^{-1} was detected within the treated area one hour after application of 2,4-D (184 g ae ha^{-1}) when a small plot of water hyacinth was treated in the La Plata River, Puerto Rico (Rodriguez and Lebron 1982). Although these studies suggest limited product deposition into the water column, little is known of aquatic herbicide deposition patterns across other scenarios such as plant growth patterns, plant architecture, herbicide chemistries, application techniques, etc. (Mudge et al. 2021).

TRACER DYES: An intuitive and effective way to better understand the interception and dispersion of herbicide applications is by adding an inert tracer substance to the spray solution and assessing its distribution or concentration fluxes as the material moves across the surface or through a column of water (Legleiter et al. 2019). Radioisotopes (e.g., tritium) were the standard tracer in the first half of the twentieth century, however, their use waned due to concerns over long-term health impacts and the training and handling requirements associated with using these unstable energy sources (Wilson et al. 1984). Although, organic compounds and dyes (i.e., fluorescein) and the colorimetric method was first documented in the early 1900s (Dole 1906) they did not become the preferred tracer until advancements in the 1950s-60s (Dybing and Currier 1959; Wilson et al. 1984). Pritchard and Carpenter (1960), Drew (1968), White (1967), and Smart and Laidlaw (1977) made significant advancements in fluorescent dye applications (e.g., use of rhodamine B, improvements in minimum detectability, and advancements in spectrophotometric techniques). Since then, tracer dyes have gained popularity and have been used across a wide range of agricultural, aquatic, food safety, and environmental applications. Although there are hundreds of commercial fluorescent dyes and colors available, most are selected for inclusion in studies based on several criteria, including (1) stability (both in sunlight and water), (2) solubility, (3) detection level (strongly fluorescent and at wavelengths not common in background materials), (4) toxicity, (5) cost, and (6) influence on physicochemical properties (Alves et al. 2014; Gao 2019; Wilson et al. 1984). Table 1 provides a list and properties of common dyes used in tracer dye research.

Table 1. The name, color index, and emission wavelength of common tracer dyes (Cai and Stark 1997; Farrugia et al. 2011; Aley 2015; Jones et al. 2019; Jiao et al. 2020).

Common Name	Color (Index Number)	Emission Wavelength Range (nm)
Brilliant Sulfoflavine	Yellow (56205)	385 to 509
Eosine	Red (45380)	516 to 538
Pyrene Tetrasulfonic	Yellow (unknown)	388 to 407
Rhodamine WT	Red (Acid Red	554 to 580
Rhodamine B	Red (45170)	542 to 580
Sodium Fluorescein	Yellow (45350)	491 to 512
Sulforhodamine B	Red (45100)	580 to 584

For many decades, particle drift and off-target movement of fertilizers and herbicides have been a major concern of the agriculture industry (Foster et al. 2018). Consequently, the industry has been on the forefront of tracer dye research and applications, using them to assess impacts on water quality (Mines et al. 2009) and biological integrity (Mull 1993). Due to their low cost, detectability, and ease of use, water-soluble tracer dyes have been used in place of formulated active herbicide and pesticide ingredients to evaluate chemical delivery technologies and off-target movement across a wide range of agricultural applications (De Schampheleire et al. 2008; Gao 2019; Menger et al. 2020; Mines et al. 2009; Rhee and Ahn 2000). Tracer dyes have also played an important role in monitoring and analyzing stream and open-water hydrodynamics. Specifically, fluorescent dyes have been used to quantify flow velocities and longitudinal dispersion coefficients, as well as estimate transport and travel times of contaminants (Abrantes et al. 2018; Julínek and Říha 2017; Kargar et al. 2020; Kilpatrick and Wilson 1989; Runkel 2015; Wilson et al. 1986).

RWT is an inert fluorescent dye that has been used as a suitable tracer of herbicide spray deposition in foliar and soil applications in terrestrial settings (Everts and Kanwar 1994; Barber and Parking 2003; Roten et al. 2013; Foster et al. 2018). RWT has also been the standard for evaluating a wide range of water exchange processes, from characterizing the hydrodynamics of wetland systems (Runkel 2015), to monitoring submersed aquatic herbicide treatments (Fox et al. 1991a; Fox and Haller 1992; Getsinger et al. 2013; Koschnick et al. 2010). Tracer studies in aquatic systems are typically performed using conservative tracers such as RWT because their composition is not attenuated by biophysical processes in surface water applications (Legleiter et al. 2019). RWT dye is also compatible with and has been correlated to the dissipation of the aquatic herbicides diquat, endothall, fluridone, and triclopyr when tank mixed and applied subsurface to target invasive submersed aquatic vegetation (Fox et al. 1991b, 1993, 2002; Langeland et al. 1994; Turner et al. 1994). Dye concentrations have traditionally been measured *in situ* by grab sample, handheld fluorometers, or estimated with Lagrangian models to achieve an accurate, consistent, rapid, and cost-efficient analysis at lower detection limits, when compared to the herbicide alone (Nelson et al. 2018). These dyes can also be more stable than some aquatic herbicides when applied to the foliage and subsurface, and result in no phytotoxicity to vegetation. This is important when using RWT for water exchange tracing since plants remain intact during application and do not lose integrity, which could artificially increase water flow. The dye also possesses no herbicidal properties during foliar deposition modeling, so plant material remains healthy and deposition patterns can be measured throughout the research.

Fluorometers are a cost-efficient way to monitor herbicide movement when tank mixed with fluorescent dye and are used to measure the intensity of fluorescent light in a sample, which is proportional to the amount of fluorescent substance present (Wilson et al. 1984). Recent studies by Mudge et al. (2021) and Sperry et al. (2022) show positive results using tracer dyes and fluorometers to measure spray distribution and concentrations in a mesocosm setting. Whenever RWT was used as a surrogate for aquatic herbicides, in-water spray deposition fractions decreased as waterhyacinth (*Eichhornia crassipes*), waterlettuce (*Pistia stratiotes* L.), and giant salvinia (*Salvinia molesta*) density increased (Mudge et al. 2021). In general, carrier volumes ≤467 L ha^{-1}, resulted in less spray loss to the water column across the same floating species. Also, mesocosm trials were conducted to determine the influence of spray trajectory angle, spray pattern type, and spray method on spray loss when applied to floating plants (B. Sperry unpublished data). For example, a forward spray trajectory angle (90°) resulted in 22% less spray loss in waterhyacinth compared to downward (90°) and forward (45°) spray trajectory angles. During these mesocosm trials no differences were detected between broadcast boom and spray-to-wet hand-gun application techniques to either waterhyacinth or waterlettuce. However, RWT spray loss and retention varied across floating species and spray pattern types when comparing single nozzle cone, single nozzle straight stream, and multi-nozzle broadcast boom.

Despite the success and ease of using RWT as an herbicide surrogate in small-scale settings, capturing data in a field setting presents challenges, particularly in large, continuous surface mats of plants (i.e., waterhyacinth and giant salvinia). Collecting these data (herbicide alone and in combination with dye) by grab sample or handheld instrumentation can be both time and labor intensive, and due to their discrete nature, they can miss areas where concentrations are present but not measured. It can also be challenging to gather RWT data in a field setting when it is critical to not disturb the plants or water surface, or when rapid data collection prior to dye dissipation into the water column is required. There is currently a need to fill significant method, data, and knowledge gaps to better understand factors that influence herbicide spray loss or retention and aid in more efficient field data collection (especially in large and hard to access sites), thus reducing uncertainty related to chemical applications and impacts.

REMOTE SENSING: Remote sensing can provide an unobtrusive detection of fundamental biological, chemical, and physical information (e.g., location, elevation, biomass, temperature, moisture) about features and processes on the Earth's surface by use of satellite or aircraft. Significant advantages of remote sensing include the ability to collect data across a wide range of spatial, spectral, and temporal resolutions in high-risk situations and in inaccessible or difficult to access areas, in which traditional survey methods may be challenging. The detection and quantification of surface features and processes via air- and space-borne data is a standard approach for assessing expansive areas where ground-based survey methods may be costly, time-prohibitive, or limited (Adam et al. 2010; Klemas 2013; McCarthy et al. 2015). Previous studies have utilized high spatial, spectral, and temporal resolution imagery, ground verification data, and a combination of remote sensing techniques (e.g., spectral indices and supervised/unsupervised classifications) to provide perspectives on systems and processes that historically have been difficult to evaluate (Anderson and Gaston 2013; Keim et al. 2013; Rapinel et al. 2014; Lane et al. 2015; McCarthy et al. 2015).

Remote sensing systems consist of a range of platforms (i.e., ground-, air-, water-, and space-borne) which carry a sensor or suite of sensors. Sensors fall into two primary categories: passive and active. Passive sensors record electromagnetic radiation reflected or emitted from the Earth's terrain – where the Sun acts as the source of energy (Shippert 2004). Active sensors provide their own electromagnetic energy, emitting those energies onto the target (i.e., features on the Earth's surface) and recording the amount of radiant flux scattered back to the sensor, or the time it takes for the energy to return (Campbell and Wynne 2011; Jensen 2009). Sensors are also categorized by their spatial, spectral, and temporal resolutions. Spatial resolution is a measure of the smallest linear or angular separation between two objects that the sensor can distinguish and is generally a function of the sensor's altitude (Jensen 2009). Spectral resolution is a sensor's ability to distinguish wavelengths of energy within the electromagnetic spectrum and is dependent upon the number of spectral bands (e.g., multispectral, 3–10 bands with relatively large bandwidth, 20–40nm; or hyperspectral, hundreds of bands with narrow bandwidths, 2–5nm), their wavelength location (e.g., visible, infrared, and microwave regions), and the narrowness of their bands (Lillesand et al. 2015). Temporal resolution is the time-step or frequency at which a sensor records data of a particular area (Jensen 2009).

Remote Sensing in Invasive Plant Management. Traditionally, nuisance vegetation communities have been surveyed and mapped via field-crew-based reconnaissance. Given the extent of water features and vegetation distribution throughout aquatic systems, as well as the time, resources, and cost-intensive nature of field reconnaissance, field-based methods are often limited. Additionally, field surveys of aquatic vegetation communities are often impeded by access restrictions (Vis et al. 2003). However, these problems may be overcome with the use of remote sensing applications. Previous studies have demonstrated the ability of remote sensing data and methods to identify and quantify aquatic vegetation type and distribution (Allen and Suir 2014; Jensen et al. 1992; Steeves et al. 1999). These remote sensing methods are increasingly important to nuisance aquatic plant management programs because they (1) reduce the time and resources required for mapping aquatic plant infestations; (2) provide information that is critical for the planning, monitoring, and effectiveness of vegetation removal efforts (Jakubauskas et al. 2002); and (3) provide capabilities for predicting future aquatic plant infestations and identifying areas of concern (Suir et al. 2018).

Previous remote sensing systems have likewise had their limitations, often associated with inadequate spatial, spectral, or temporal resolutions. However, recent advancements in remote sensing systems (i.e., improvements and miniaturizations of electronics, navigation, and telemetry components), platforms (e.g., UAS), sensors (e.g., machine vision cameras), data (e.g., hyperspectral imagery [HIS]), and techniques (e.g., machine learning and data fusion) provide flexible systems and products that, in many cases, overcome previous constraints and therefore, have the potential to supplement or maximize traditional field-based techniques (Herwitz and Hobbs 2006; Whitehead and Hugenholtz 2014). These advancements in remote platforms, sensors, and data processing also provide novel metrics and tools for assessing and monitoring changes in aquatic vegetation communities and landscape patterns, and, by extension, the impacts from and effects on system drivers, underlying ecological processes, or control measures (Kupfer 2012). These tools and techniques can ultimately provide capabilities for assessing significant biophysical and ecological parameters, monitoring the short- and long-term impact or effectiveness of management activities, and predicting location and severity of future aquatic nuisance plant infestations (Thiago et al. 2008; Bourgeau-Chavez et al. 2009; DigitalGlobe 2010; Allen and Suir 2014; Bourgeau-Chavez et al. 2015).

Use of UAS and Tracer Dyes. UASs are defined as all elements (i.e., craft, payload, and all components that control them) that are required to operate an unmanned aircraft safely and efficiently in the national airspace system (FAA 2016). UASs consist of a flying device that includes (1) a frame or platform with the structural, mechanical, and electronic elements necessary for the flight, its control, and stability; (2) a set of sensors and devices for the acquisition of information from the environment; and (3) a ground control station (Vélez-Nicolás et al. 2021). Recent advancements in UASs (i.e., technological and operational) have resulted in cost and risk reductions, increased efficiencies (e.g., operating at low altitudes and closer to targets), enhanced products, and improved perspectives on projects (Association of Governmental Risk Pools [AGRiP] 2015; Herwitz and Hobbs 2006; Ma et al. 2013). Due to these advancements, UASs are emerging and adaptable platforms that are demonstrating the ability to overcome some of the shortcomings (e.g., atmospheric distortion) and inherent difficulties (e.g., areal extent and access) associated with other field- and remote-based methods (Whitehead and Hugenholtz 2014).

The spatial, spectral, and temporal advantages afforded by UASs and their derived products can be substantial for monitoring and quantifying ecological processes and ecosystem services and are therefore becoming more commonly used in the study of forested, fluvial, and aquatic systems (Bagaram et al. 2018; Brouwer et al. 2015; Getzin et al. 2012; Kent et al. 2015). These UAS advantages have also been exploited in the study of river morphology, such as channel evolution, bedform migration, bank erosion, and bathymetry mapping, as well as with key hydrodynamic processes including river stage fluctuations, water budgets, river discharge, surface velocity, flooding, and temperature mapping (Vélez-Nicolás et al. 2021). Many of these hydrodynamic processes, albeit without resolving vertical structure, are now being studied using relatively simple and low-cost systems that incorporate UAS-mounted passive sensors and fluorescent dyes (Clark et al. 2014). Some studies have used fluorescent dyes (e.g., fluorescein and RWT) and UAS-collected multispectral imagery to map plume concentration profiles, construct concentration curves and mathematical dispersion models, and compared those to *in situ* samples collected using fluorometers (Burdziakowski et al. 2021; Powers et al. 2018). For instance, Baek et al. (2019) used a UAS with a standard commercial digital RGB (red, green, blue) camera, *in situ* fluorometric probes, and artificial neural network models to correlate ($R^2 > 0.9$) image digital numbers to RWT dye concentrations in open channels. Other studies have used UASs, tracer dyes (as surrogates for hazardous agents), and field measurements to map and quantify oil and harmful algal bloom concentrations and plumes in lake and coastal ocean environments (Duan et al. 2019; Filippi et al 2021; Pokrzywinski et al. 2022; Johansen et al. 2022).

UASs and tracer dyes have also experienced increased utilization in applying and studying pesticides for insect, disease, and nuisance plant control (Sarghini et al. 2019). Until relatively recently, tracking herbicide spray distribution and movement using fluorescent dyes were accomplished primarily with *in situ* sensors or collectors, which can be a difficult task (Richardson 1984). Recent studies by Qin et al. (2018) and Wang et al. (2020) have shown the utility of UASs for applying herbicides and/or assessing spray deposition, pattern, long-distance transport, and drift potential of fluorescent dye spray applications (as surrogates for herbicides and pesticides). UASs also provide the spatial and temporal resolutions that can be helpful for evaluating small-scale features and dynamics, which can be useful to better understand tracer dye (herbicide) dispersion patterns.

Although standard remote sensing systems can provide advantages over field-based methods for evaluating dye distribution and concentration, they can also be limited, especially with dense vegetation environments or in complex or turbid water systems. Fortunately, there are several sensors, models, techniques, and tools that are advancing UAS applications. For instance, Legleiter et al. (2019) demonstrated how UAS-collected hyperspectral imagery, an optimal band ratio analysis (OBRA) algorithm, and spectral mixture analysis (SMA) techniques can be used to estimate dye concentrations more accurately across a range of turbidity levels, over a large spatial-area, and long-duration. Spectral mixture analysis, which provides a means of quantifying subpixel information, can be an important tool for inferring the presence of varying amounts of tracer within a pixel (Legleiter et al. 2022; Malhi et al. 2020). Others, like Teickner at al. (2019) have used UASs and machine learning algorithms, like support vector machines (SVM), to semi-automate the detection and identification of fluorescent dyes. In addition to these recent developments, there are novel remote sensing applications being applied in other fields of research that have potential in UAS-based fluorescent dye research. One such application is the use of machine vision cameras, which have significantly more sensing elements than traditional remote sensors and have the capacity to automate color verification (Balaban et al. 2005). Although automated color verification via machine vision cameras has been used primarily for the inspection of manufactured goods (Chauhan et al. 2011), it is theorized that a similar method could be useful in fluorescent dye detection and concentration estimation. It is also theorized that a UAS that incorporates narrow bandpass filters (those matching the emission wavelength of the fluorescent dye) with machine vision cameras could provide an automated system capable of detecting dye presence and dye concentrations in real-time.

CONCLUSIONS: A literature review has revealed a need to develop and/or test novel remote sensing methods for detecting the presence and concentration of tracer dyes (as a surrogate for herbicides) applied in aquatic systems. The use of UASs and fluorescent dyes to detect and quantify herbicide spray distribution is a budding science, and there are still significant gaps in our knowledge and analysis capabilities. Future studies should assess the use of remotely sensed data and products to help evolve the science of herbicide treatment. In addition, future research should evaluate a range of sensors, dyes, filters, and methods across various aquatic conditions (e.g., range of turbidity and vegetation density) and spray applications. Evaluations should include air- (manned and/or unmanned aircraft systems) and space-borne platforms; machine vision cameras, and multispectral, and hyperspectral sensors; as well as potential benefits of band ratioing or data fusion for the development of new metrics or algorithms.

It is anticipated that with recent and future advancements, remote sensing used in conjunction with tracer dyes will help improve chemical application monitoring and efficiency, and ultimately reduce the level of uncertainty related to non-target impacts. In addition, combining UAS, dye, and herbicide technologies can aid in training aquatic applicators (novel or veteran), in monitoring plant coverage, and in determining if spray drift moves off-target or off-site, where it can impact native or threatened and endangered species.

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