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Research Article

A dip or a dab: assessing the efficacy of Virasure® Aquatic disinfectant to reduce secondary spread of the invasive curly waterweed *Lagarosiphon major*

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Abstract

Aquatic alien invasive species (AIS) are a substantial and increasing threat to biodiversity and ecosystem function worldwide. In particular, invasive aquatic macrophytes, such as the South African curly waterweed *Lagarosiphon major* ((Ridley) Moss 1928), induce major environmental change that often culminates in wide-ranging ecological and socio-economic impacts. Currently, there is a lack of effective biosecurity protocols to mitigate against such invader spread. Here, we examine the efficacy of a broad-spectrum aquatic disinfectant, Virasure® Aquatic, to induce mortality of *L. major* propagule stages. We assessed the efficacy of Virasure® Aquatic at contact times of 1, 2 and 5 minutes, using 1% (10g L^{-1}) and 4% (40g L^{-1}) concentrations. A necrosis scale was applied to visually assess tissue degradation. Necrosis increased with longer chemical contact times, with fragment degradation optimised at 2 minutes at 1% concentration and 1 minute at 4% concentration. Mode of application was also critical to treatment effectiveness, with spray treatments less effective than submersion treatments. We recommend the use of Virasure® Aquatic *via* submersion for a minimum period of 2 minutes at 1% concentration or higher. While spray applications should be applied when submersion is not feasible, such as with large water craft, increased spray times beyond those assessed here should be examined. However, results presented suggest that Virasure® Aquatic can effectively reduce the secondary spread of invasive *L. major*, and may thus form an integral part of biosecurity protocols. The use of broad-spectrum disinfectants and other readily available treatments, that were not purposefully developed for aquatic AIS control but nevertheless are emerging as effective in aquatic AIS management, is discussed and encouraged.

Key words: biosecurity, aquatic disinfectant, invasive species management, potassium peroxymonosulfate, spread prevention, macrophyte

Introduction

Aquatic alien invasive species (AIS) are considered a major driver of adverse change to freshwater ecosystems (Simberloff et al. 2013; Piria et al. 2017). In particular, many invasive aquatic plants (especially invasive macrophytes) detrimentally affect freshwater community dynamics and ecosystem function *via* negative alteration of biotic and abiotic conditions (Schultz and Dibble 2012; Hussner 2014; Kuehne et al. 2016). In addition, the considerable biomass associated with the presence of large monospecific swards of invasive macrophytes can inhibit many recreational and commercial activities, increase flooding frequency, and result in substantial economic costs (Williams et al. 2010; Lafontaine et al. 2013). Novel methods for invader eradication and control, which balance efficacy with cost, legislative barriers and non-target effects, are thus urgently required.

Despite a restricted ability to self-disperse, many aquatic AIS continue to successfully invade hydrologically unconnected sites (Hussner 2012; Caffrey et al.

2016; Coughlan et al. 2017a). While vectors that underpin the natural dispersal of aquatic AIS are often not fully determined (Coughlan et al. 2017b), freshwater systems remain highly vulnerable to accidental invader introductions due to their interconnectedness and exposure to multiple transport vectors, e.g. angling and boating (Rothlisberger et al. 2010; Banha et al. 2016). To date, various stakeholder biosecurity campaigns (e.g. "Check, Clean, Dry") have attempted to reduce the spread of aquatic AIS (Anderson et al. 2015) by creating awareness and endorsing best practice. Moreover, recent European Union (EU) legislation (Regulation 1143/2014) requires EU Member States (MS) to enforce rapid control, spread prevention and eradication of damaging invaders that are listed as Invasive Alien Species of Union Concern. Furthermore, recent United States of America (USA) legislation (Safeguarding the Nation from the Impacts of Invasive Species – amendment to Executive Order 13112) seeks to prevent, control and eradicate invasive species.

Currently, management options for the eradication and control of established invader populations are often complex, resource-intensive and costly, and achieve only limited success (Hussner et al. 2017; Piria et al. 2017). Indeed, there are relatively few examples of successful reductions and/or eradications where invaders have already established (Hussner et al. 2017). As prevention of aquatic AIS introductions is the most economical way to safeguard ecosystems, the development of efficient and cost-effective biosecurity protocols that prevent invader spread is essential (Barbour et al. 2013; Simberloff et al. 2013; Caffrey et al. 2016; Hussner et al. 2017; Coughlan et al. 2018a). Presently, however, there exists only a limited understanding of the relative efficacies of various biosecurity measures (Barbour et al. 2013; Anderson et al. 2015; Piria et al. 2017; Coughlan et al. 2018a). Chemical treatment has been suggested as a suitable mechanism to control aquatic AIS spread, as this is often more economical and widely applicable when compared to other methods (Getsinger et al. 2008; Richardson et al. 2016). However, chemical treatments have hitherto been predominantly applied *in situ* where invasive populations have already established (e.g. glyphosate, Emerine et al. 2010; metsulfuron, Clements et al. 2014), often with inconsistent rates of success (see Hussner et al. 2017). Thus, innovative measures to reduce invasive species spread are urgently required (e.g. Coughlan et al. 2018b). While alternative broad-spectrum aquatic disinfectants may prove effective at reducing secondary spread of invaders, these chemicals have yet to be thoroughly considered as aquatic AIS biosecurity agents.

Lagarosiphon major ((Ridley) Moss 1928) is a canopy-forming submerged invasive macrophyte, native to South Africa (Caffrey et al. 2010). In the Northern Hemisphere, *L. major* displays over-winter growth and can achieve substantial biomass under conditions that are unsuitable for many native species, including within eutrophic waters (Martin and Coetzee 2014). Despite being listed as an EU Invasive Alien Species of Union Concern, *L. major* is still commonly sold as an oxygenating plant for aquaria and artificial watercourses. Like many invasive macrophytes, *L. major* predominantly reproduces and spreads by vegetative propagation, particularly *via* vegetative fragments which have been observed to exhibit a high survival potential (Redekop et al. 2016; Coughlan et al. 2018a). Moreover, given the high level of fragmentary propensity associated with *L. major*, propagules are commonly observed to be transferred *via* boat motors and fishing nets (Matthews et al. 2012).

Here, we assess the efficacy of Virasure® Aquatic as a biosecurity agent to reduce the secondary spread of *L. major* fragments under varied chemical concentrations, exposure times and modes of application. While aquatic disinfectants had previously been developed for applications outside of invasive species management, several have been observed to effectively and rapidly induce aquatic AIS mortality (e.g. Virkon®/Asian clam; Barbour et al. 2013), but none have been previously tested upon invasive macrophytes.

Methods

Cultivation of Lagarosiphon major

Shoot portions of *L. major* were harvested from an artificial pond at Greenacres Golf Centre, Ballyclare, Northern Ireland (N54º43′28.631; W06º00′10.908), between February and April 2016. Substrate was also sampled from the collection site for use in laboratory cultures and the experiment using a spade. Vegetative samples were rinsed and transported to Queen's University Belfast in dechlorinated tap water. *Lagarosiphon major* was maintained in continuously aerated aquaria within the laboratory at 13 ± 2 °C under a 12:12 light:dark regime. All plants were acclimatised for one week prior to experimentation.

Efficacy of Virasure® Aquatic as a biosecurity agent for Lagarosiphon major

Healthy apical shoot sections of *L. major* were selected for submersion and spray treatments with Virasure® Aquatic (Fish Vet Group, 22 Carsegate Road,

Table 1. Scale describing visual tissue degradation stages of *Lagarosiphon*.

Scale	Description
0–10%	Tissue degradation at site of fragmentation
10–20%	Pale brown leaf at apical tip
20–30%	Pale brown leaf ends anywhere on plant
30–40%	All leaf ends pale brown
40–50%	Fragment collapse < 90°
50–60%	Full leaves pale brown
60–70%	All full leaves pale brown
70–80%	Fragment collapse ≥ 90°
80–90%	Full leaves dark brown/fragmenting at tips
90–100%	Full fragment degradation: leaves fragmented and dark, flattened against stem

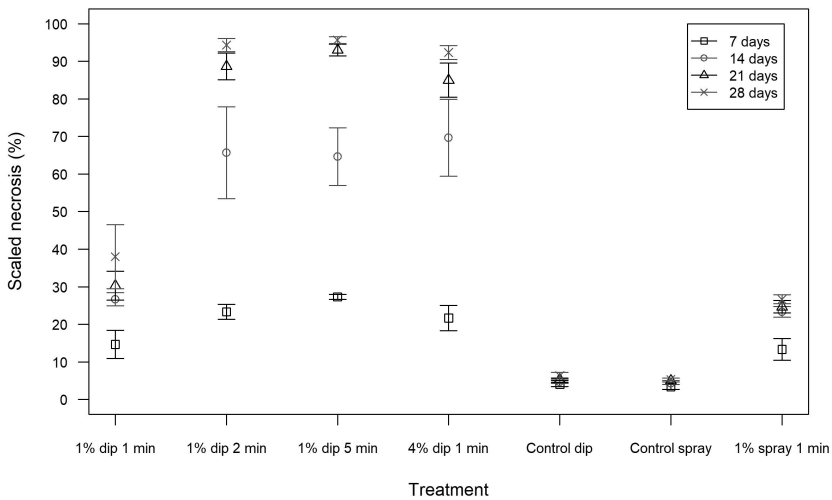

Figure 1. Mean (± SE) necrosis of *Lagarosiphon major* propagule fragments over a 28 day period post-exposure to 1% Virasure® Aquatic for 1, 2 and 5 minutes *via* submersion; to 4% Virasure® Aquatic for 1 minute *via* submersion; and to 1% Virasure® Aquatic for 1 minute *via* continual spray, alongside control submersion and spray treatments (n = 3).

Inverness, Scotland, IV3 8EX). A necrosis scale was developed to monitor tissue degradation following the various exposure treatments (see Table 1). Individual 5 cm apical fragments of *L. major* were either submerged in a 1% Virasure® Aquatic (10 g L^{-1}) solution for 1, 2 or 5 minute exposure, or were submerged in a 4% Virasure® Aquatic (40 g L^{-1}) solution for 1 minute exposure. Control samples were submerged in dechlorinated tap water for 1 minute. Other 5 cm fragments were concurrently sprayed continually with 1% Virasure® Aquatic solution for 1 minute, while control samples were sprayed with dechlorinated tap water for 1 minute. All treatments were replicated three times. Following treatment, each fragment was individually submerged in dechlorinated tap water in 0.15 litre cylindrical containers measuring 8 cm diameter, with sufficient substrate to cover the basal area to monitor recovery. Further, comparative photographs were taken weekly to support visual estimation of tissue degradation. All experiments were conducted in a randomised design.

Statistical analyses

All data analyses were undertaken in R version 3.4.2. (R Core Team 2017). We analysed categorically scaled necrosis (Table 1) with repeated measures using ordinal logistic regression. Experimental observations for treatment effectiveness spanned 28 days at weekly intervals, with explanatory variables "treatment" and "time" incorporated as both single and interacting terms in the model. Tukey's comparisons were used to perform *post hoc* analyses where terms yielded significance.

Results

Minimal fragment degradation was observed for both control treatments (Figure 1; Figure 2). However, Virasure® Aquatic significantly increased fragment tissue degradation (χ^2 = 73.46, df = 6, P < 0.001; Figure 1). Even submergence or spraying for 1 minute in 1% Virasure® Aquatic resulted in significant fragment degradation (submergence, z = 5.89, P < 0.001;

Figure 2. *Lagarosiphon major* propagule fragments at 7 (A), 14 (B), 21 (C) and 28 (D) days post-treatment with Virasure® Aquatic. Top row (L–R): control submerged 1 minute, submerged 1 minute (1%), submerged 2 minutes (1%). Middle row (L–R): submerged 5 minutes (1%), control spray 1 minute, spray 1 minute (1%). Bottom: submerged 1 minute (4%). Photographs by RNC.

spraying, $z = 5.86$, $P < 0.001$). While there was no difference between submergence or spray treatments for 1 minute with 1% Virasure® Aquatic ($z = 1.50$, $P = 0.74$), submergence for 2 minutes in 1% Virasure® Aquatic was significantly more effective than both 1 minute submergence ($z = 9.56$, $P < 0.001$) and spraying ($z = 11.79$, $P < 0.001$). However, there was no significant difference between 2 minute submergence and 5 minute submergence in 1% Virasure® Aquatic ($z = 1.08$, $P = 0.93$), nor when compared to 1 minute submergence in 4% Virasure® Aquatic (2 minutes, $z = 0.19$, $P = 0.99$; 5 minutes, $z = 1.20$, $P = 0.89$).

Overall, necrosis increased with time after treatment ($\chi^2 = 86.82$, $df = 3$, $P < 0.001$; Figure 2). There was a significant difference between degradation observed between all incremental observation periods (all $P \leq 0.002$). There was a significant "treatment × time" interaction ($\chi^2 = 98.82$, $df = 18$, $P < 0.001$), which reflected the relatively rapid attainment of full degradation with longer exposure time and greater chemical concentration, whilst controls survived (Figure 1). Although not accounted for quantitatively, regrowth of shoots was observed within control fragments and fragments treated with 1% Virasure®

Aquatic *via* spray only, indicating sustained fragment viability of control and spray treatments, but not submersion treatments (Figure 2).

Discussion

Aquatic alien invasive species (AIS) continue to spread at unprecedented rates, reducing biodiversity and altering ecosystem function (Seebens et al. 2017, 2018). As aquatic ecosystems are highly susceptible to aquatic AIS introductions, the identification and integration of cost-effective and widely-applicable protocols to reduce invader spread is essential. Invasive aquatic plants have exerted particularly profound negative impacts on recipient ecological communities (Schultz and Dibble 2012; Hussner 2014; Kuehne et al. 2016). Virasure® Aquatic can induce substantial necrosis, morbidity and mortality of *L. major* fragmentary propagules. Accordingly, biosecurity protocols can likely be improved with the use of this broad-spectrum aquatic disinfectant. To date, research has largely focused on the efficacy of Virkon® Aquatic, a similar aquatic disinfectant, to control aquatic AIS such as Asian clam, *Corbicula fluminea* (Barbour et al. 2013), quagga mussel, *Dreissena rostriformis bugensis* (Moffitt et al. 2015), and the gastropod red-rimmed melania, *Melanoides tuberculata* (Mitchell et al. 2007), all under varying exposure times. Treatment of submerged plants using herbicides is strictly prohibited across Europe due to adverse environmental impacts, thus inherently restricting management options for aquatic AIS (Hussner et al. 2017). Accordingly, the use of aquatic disinfectants outside of water, to reduce secondary spread of invaders, is pertinent, timely and more environmentally friendly.

A 2 minute submersion using 1% Virasure® Aquatic solution can achieve full *L. major* fragment degradation. However, longer exposure times and greater chemical concentrations will likely increase the rapidity of fragment mortality. When submersion is not feasible, a spray treatment should be applied using longer contact times than assessed within the scope of the present study, and a higher concentration (≥ 4% solution) should be applied. The limited efficacy of shorter submersion in, or spraying treatments with, 1% Virasure® Aquatic solution may result from a lack of adherence to plant tissue. These results corroborate with those of Paetzold and Davidson (2011), wherein an invasive sea squirt was found to be largely unaffected by spray treatments with Virkon® Aquatic.

In the present study, we examined relatively large plant fragments as these are known to exhibit a greater capacity for regrowth (Wu et al. 2007; Jiang et al. 2009), and also reduce inhibition of lateral growth driven through apical dominance (Cline 1991). Yet, the size of fragments examined is still likely within the threshold of propagules which are readily entangled with, and transported overland by, anthropogenic vectors (Barrat-Segretain et al. 1998). The lack of root growth observed here may be a result of the timescale permitted, poor anchorage, or potential apical dominance of samples (Cline 1991; Wu et al. 2007). Critically, our results demonstrate that the application of Virasure® Aquatic can induce substantial and complete degradation of *L. major* propagules. The working pH for the main oxidising ingredient (potassium peroxymonosulfate) of Virasure® Aquatic is strongly acidic (2.6) when diluted in a 1% solution at 20 °C, facilitated through the presence of two organic, malic and sulphamic, acids (see Fish Vet Group 2015). However, *L. major* may only be particularly susceptible to these compounds due to the species' characteristic tendency to induce and tolerate high levels of alkalinity (Stiers et al. 2011). Furthermore, negligible toxicities to non-target vertebrate species following short-term exposure to a compositionally-similar aquatic disinfectant have been demonstrated (Stockton-Fiti and Moffitt 2017), and therefore the focal product may be safe for use proximal to water.

Our promising results suggest further experimental examination of the efficacy of aquatic disinfectants to reduce aquatic AIS spread to be critical. Accordingly, additional trials investigating the impacts of such chemical solutions on aquatic AIS propagule stages should be considered, alongside assessments for potential non-target effects on other native species, particularly macroinvertebrates associated with aquatic macrophytes. Further aquatic disinfectant efficacy examinations towards other existing and emerging floral and faunal aquatic AIS are urgently required. Disinfectant trials should concurrently seek to examine different contact times, recovery conditions and varied chemical concentrations upon a variety of anthropogenic vectors, such as equipment associated with angling and boating, in order to maximise the transparency of results. Finally, the incorporation of aquatic disinfectants within biosecurity management protocols requires urgent consideration by stakeholder groups.

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Author contributions

RNC, KC, JMC and JTAD designed the study; RNC conducted the experiment and data analysis; RNC produced the first draft of the manuscript; all authors contributed to writing the manuscript, which was led by RNC.

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