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Croft, Evan M. ***Non-chemical European buckthorn (Rhamnus cathartica) removal effectiveness and costs compared to chemical removal methods***

Abstract

Common or European buckthorn (*Rhamnus cathartica)* and glossy buckthorn (*Frangula alnus)* are invasive species that can take over forest floors, alter soil properties, and out-compete native species for sunlight, nutrients, and water. Buckthorn is capable of assisting the cultivation of potential invasion meltdowns across the midwestern U.S. and southern Canada via increasing decomposition of ground litter, altering the carbon-nitrogen ratio in soils by increasing the nitrogen level, facilitating other species' invasions, and shading out seedlings of keystone species. Buckthorn resprouts readily from roots and stumps, requiring laborious mechanical removal or potentially costly chemical treatment. A thorough review of the process, costs, effectiveness, and environmental impact that are associated with the removal of buckthorn has reinforced the need for finding a non-chemical control method that is similar in its ability to kill an individual buckthorn to its chemical counterparts. I investigated the cost and effectiveness of buckthorn removal using Buckthorn Baggies applied to cut stumps at two locations in Wisconsin. Buckthorn Baggies successfully killed 80.7% of buckthorn over the treatment period (95% CI: 70.8-87.8%), and effectiveness decreased with increasing stem width ($z = 3.26$, $p = 0.001$). Costs for Buckthorn Baggies average slightly higher than chemical removal methods. Buckthorn Baggies provide an environmentally safe way to remove buckthorn effectively. Success is dependent on stem width but may be dependent on other factors. Future research on Buckthorn Baggie effectiveness will need to be directed towards additional factors that may influence success rates

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Chapter I: Introduction

European buckthorn (*Rhamnus cathartica)* and glossy buckthorn *(Frangula alnus* or *Rhamnus frangula L.)* are prolific, highly invasive species that have sparked interest in the field of conservation biology and land management. There have been multiple studies that indicate *R. cathartica* is responsible for facilitating invasional meltdown in ecosystems by creating litter layer and soil condition alterations that are favorable for other invasive species (Heimpel et al., 2010). Buckthorn assist in providing a habitat that is suitable for European or common earthworms (*Lumbricus terrestris*), and they provide overwintering protection for oat crown rust (*Puccinia coronate*) and soybean aphids (*Aphis glycines*), which can facilitate ground beetle and lady beetle infestations (Heimpel et al., 2010). Successful management of *R. cathartica* and *F. alnus* will require an understanding of their basic characteristics, history, interactions with other species, chemical control methods and costs, and non-chemical control methods and costs.

The purpose of this study is to investigate an alternative buckthorn removal method that helps keep the already altered soil dynamics intact by omitting chemical use, stump grinding, and pulling of stumps that can further loosen soil. Buckthorn Baggies are a non-chemical buckthorn removal method that was created in Minnesota by John Hamilton. Buckthorn Baggies aim to take the place of chemical application in the field that can be harmful to native species and water quality. This ongoing study takes place in Stanley, WI and at Sportsman Lake in Owen, WI. The Stanley site location is my residential home, purchased February 2021. May 2021 revealed an alarming number of buckthorn planted along the south and east property lines. Sportsman Lake is an artificial lake that is owned by Clark County Land Conservation Committee (CCLCC). With permission from CCLCC, a survey was done for buckthorn, which indicated a need for removal. Sportsman Lake is currently facing runoff issues and increased algae issues. Individual

buckthorn in both locations were cut 16 cm above the soil surface and covered by Buckthorn Baggies following the manufacturer's instructions in August 2021. The bags were removed in March 2022, 7 months after initial treatment, and the stumps were assessed for survival. I then used logistic regression to model buckthorn survival as a function of site and stem width.

I compared the cost and effectiveness of Buckthorn Baggies to six chemical treatment methods. Each herbicide label specifically prohibits application near water, areas below high-water marks, or wetlands (Pergams & Norton, 2006). Issues with chemical methods involve a lot of surveying, marking, and spraying errors, where in some cases 6-21% of marked buckthorn shrubs were missed during application. Errors in application can greatly undermine efforts for removal, especially if not properly followed up on (Pergams & Norton, 2006). Multiple studies indicate loss of non-target species with herbicide use on buckthorn (Au & Tuchscherer, 2014; Pergams & Norton, 2006; Schuster et al., 2020). Minimization of non-target species loss is a desired outcome in invasive species removal.

Non-chemical methods of cutting, girdling, and mowing are much less effective than chemical methods, but often benefit the environment from lack of chemical use. Regardless of the control method used, there will be a requirement of multiple rechecks, resprays, rebagging, and repeated treatments for years to follow in order to truly stifle the buckthorn invasion.

The assumptions of this study include that the individual responsible for determining survival can do so accurately. It is assumed that all requirements for a logistic regression model are met, with the exception of a random representative sample. The sample size of 83 includes two very localized populations with minimal differences between individuals in their own groups. This may cause the sample to fall short of this requirement. It is understood that this study does not represent all environments and ecosystems. Based on research by Pergams and

Norton (2006) it is assumed that the chemical treatment methods Tordon RTU and Stalker are equally as effective as Roundup. Finally, it is assumed that all chemical data supplied by manufacturers and industry professionals are accurate in their methods and results used in this study.

The most important take-away from this study is the environmental effects of each chemical application on soil and water, highlighting a need to find a removal method for buckthorn that can be used in sensitive waterways and wetlands that these shrubs tend to invade. In order to be truly accurate, studies would require preinvasion baseline data for soil chemistry, biodiversity of understory and hardwood stands, and any other invasive organism mentioned in this study. This is the most challenging aspect of investigating buckthorn invasion history (Iannone et al., 2015; Rooney & Rogers, 2011). There are numerous questions about the relationships between buckthorn, common earthworms, soybean aphids, allelopathy, emodin, soil composition alterations, water moisture levels, and more that need further research and more detailed studies to ensure these relationships are truly involved with one another. Lastly, all methodologies described will discuss respective errors and limitations with consideration to the complex relationship buckthorn shrubs have with the environments they invade.

Chapter II: Literature Review

Buckthorn Natural History

Buckthorn are deciduous, multi-branched shrub-like trees that can reach up to nine meters tall and are native to Europe, Asia, and Northern Africa. They prefer to grow in previously disturbed areas that allow ample light and have minimal ground litter cover that prohibits seed germination, preferably in lightly shaded deciduous forests. Buckthorn can be found residing in forest edges, old farm fields, prairies, wetlands, riverbanks, and occasionally further into forest understories. The ability of buckthorn to be more responsive to light than other shade tolerant plants is a key factor in their ability to out-compete native species for sunlight. Buckthorn are often thriving in deciduous forests, but coniferous forests and coniferous litter can stifle buckthorn invasion and reproduction success by deterring them from settling in coniferous stands. Coniferous litter is not as favored by rodents and worms for food sources and creates tightly packed layers of litter that greatly stifle seed germination rates (Delanoy & Archibold, 2006; Heneghan et al., 2004, 2006; Knight et al., 2006; Labonté et al., 2020).

R. cathartica has dark green leaves with slightly toothed edges, and three to five veins that move to the tip of the leaf. *F. alnus* has dark green leaves that are smooth-edged with a glossy coating and contain eight to nine pairs of veins. The wood from buckthorn has an orange core with a more yellow wood on the outside (Clauson, 2011; Iannone et al., 2013; Minnesota Department of Natural Resources, n.d.). There has been interest in the wood of buckthorn for its beautiful coloring among wood workers and craftsmen. The importance of this interest is it helps to get rid of the cut shrubs without having to burn or mulch the cut shrubs (Iannone et al., 2013). Buckthorn leaves appear earlier than most species in the spring and keep their leaves for an average of 58 days later than most surrounding species. Buckthorn have a higher-than-average

percent of nitrogen in their leaves throughout the growing season when compared against similar species of shrubs like black cherry (*Prunus serotina*), Bell's honeysuckle (*Lonicera bella),* and gray dogwood (*Cornus racemosa*). The high nitrogen content in their leaves is crucial information that helps to explain its relationships with many other invasive species and ecological functions. There are native shrubs in the *Sambucas* genus (*S. canadensis* and *S. racemose)* that perform similarly to buckthorn in the spring and fall. *Sambucas* leaves unfurl on average seven days after buckthorn and keep their leaves just as long (Wafer et al., 2020). This has led to studies on shrubs similar to buckthorn being able to limit buckthorn carbon gain in the spring and fall, which helps foster a biotic resistance to further invasions (Craves, 2015; Delanoy & Archibold, 2006).

Buckthorn are aggressive and prolific in their invasive environments. When left uncontrolled, buckthorn can spread to cover an average of 62.6% of the invadable habitat (Labonté et al., 2020). The average sex ratio stands at six to seven female trees for one male tree, and once the trees reach six years old, they start producing fruit (Knight et al., 2006). The fruit are small berries that start off as green and ripen to be black in the late summer. Buckthorn that are in wetlands or on riverbanks are more likely to start producing fruit earlier than six years of age (Knight et al., 2006). Each berry produced can contain two to five seeds. With an average germination rate of 85%, fruit predation by rodents and birds is a major contributing factor to the spread of *Rhamnus* from one location to the next (Archibold et al., 1997; McCay & McCay, 2008). American robins (*Turdus migratorius)* and European starlings (*Sturnus vulgaris)* are the two most documented feeders on buckthorn fruit (Craves, 2015). American robins have been observed nesting in buckthorn shrubs, however they often experience higher levels of egg predation from local mesocarnivores (Craves, 2015). Other major feeders on both species of

buckthorn include gray catbirds (*Dumetella carolinensis*) and cedar waxwings (*Bombycilla cedrorum*), while northern cardinals (*Cardinalis cardinalis*) and white-breasted nuthatches (*Sitta carolinensis)* have been documented feeding exclusively on *R. cathartica* but not *F. alnus* berries (Craves, 2015; McCay & McCay, 2008; Vernon et al., 2014).

Buckthorn Invasion

Buckthorn was introduced into North America in the early to mid-1800's for uses such as hedges, habitat enhancement, and other forestry projects (Heneghan et al., 2004, 2006; Klionsky et al., 2010; Knight et al., 2006; Pergams & Norton, 2006). Since the introduction of buckthorn to North America, they have invaded the midwestern and northeastern United States as well as the Maritime Provinces of Canada. Invasive shrubs are a global issue; however, buckthorn and other shrub invasions are a much more prevalent issue in North American temperate woodlands (Knight et al., 2006; Rooney & Rogers, 2011). Buckthorn introduction into non-native areas can be facilitated by multiple factors. The first factor is mesophication of oak (*Quercus spp.)* stands that start with fire suppression efforts that are leading to dominance shifts in populations to hardwood trees like the sugar maple (*Acer saccharum*) and black cherry (*Prunus serotina*; Schulte et al., 2011).

The second facilitation factor is predation and seed dispersal. Buckthorn in North America experience less herbivory when compared to the co-occurring native species. This is most likely due to the compound emodin, which is found in the bark, leaves, and unripened berries of buckthorn. Once the fruit is ripe and the emodin is no longer present, buckthorn depends on the birds mentioned above to eat the berries and scatter the seeds and rodents in genus *Peromyscus* to further move and disperse seeds. Deer mice *(P. maniculatus)* and white-footed mice (*P. leucopus*) regularly eat buckthorn seeds. Many mice will store the seeds and

forget about them or will be unable to consume them, which helps facilitate buckthorn invasion in neighboring stands (McCay & McCay, 2008; Knight et al., 2006).

The third factor found is habitat alteration. Habitat alteration comes in many forms such as road construction, urban development, deforestation, and agricultural development. Buckthorn invasions are influenced by the suitability of a habitat that has been altered. The continuing trends of deforestation and other habitat alterations will result in more suitable conditions for buckthorn along roads, forest edges, and increased urban presence (Endicott et al., 2017; Reinhardt et al., 2019; Ruiting et al., 2018).

The fourth factor branches off of habitat alteration, specifically for an invasive species' facilitation of other invasive species due to habitat alteration. Invasive species can alter food sources, create interspecific competition, and can alter habitat during their invasion. Examples of this include buckthorn's increase of deeper, nitrogen-rich leaf litter assisting in the potential facilitation of European or common earthworms (*Lumbricus terrestris*) and invasive soybean aphids (*Aphis glycines*), facilitating Asian ladybeetle (*Harmonia axyridis*) infestations. A final example recently studied by Baron and Rubin (2021) indicates facilitation of buckthorn is influenced by the emerald ash borer (*Agrilus planipennis*). Emerald ash borer is an invasive beetle that bores into ash trees (*Fraxinus spp.)* to reproduce, with adults emerging in 1-2 years, killing the tree over time, with each generation. The ability to induce ash tree mortality has led to canopy gaps that have been increasing the light availability and enhancing buckthorn invasion. This study is the first of its kind involving this facilitation factor (Heimpel et al., 2010; Baron & Rubin, 2021).

Buckthorn as a Facilitator

Buckthorn has many characteristics that play a role in its success. The alteration of soil properties, allelopathy, possible facilitation or encouragement of invasive invertebrate species, pest and disease transmission, and mammal interactions a play critical role in buckthorn's success in an invaded environment. The environmental interactions of buckthorn detail an intricate, ecological relationship between organisms to ensure its success.

Soil Alteration

Buckthorn is capable of altering soil chemistry, moisture, and decomposition rates. Removal of shrubs will not always facilitate regeneration of native species immediately. Nitrogen levels in the fruits and leaves of buckthorn are on average 1.6-2.1% higher than other native plant species. Carbon can increase by as much as 80% due to increased litter and increased decomposition rates. Increases in nitrogen help with recruitment success by encouraging the clearing of litter by common earthworms to reveal bare spots that assist in successful germination of buckthorn. Other changes involve significant changes in pH, ammonium (NH_4^+), gravimetric water content, and increased litter decomposition. Buckthorn's high germination rate is attributed to their ability to keep their habitat moist and suitable only for their success, to the detriment of surrounding native species (Heneghan et al., 2004, 2006; Iannone et al., 2015; Knight et al., 2006).

Removal of buckthorn could directly facilitate restoration of native flora in invaded sites over time. After removal, leaf litter depth increases in treated areas. This can be attributed to decreases in nitrogen in the litter layer, allowing for slower decomposition and more time to increase in depth. There are also results that show soil alterations that last after removal of

buckthorn, indicating a need for longer term studies on soil alteration properties of buckthorn, before and after invasion and removal (Bartowitz & Orrock, 2016; Heimpel et al., 2010).

Emodin and Allelopathy

Buckthorn has allelopathic characteristics associated with the chemical emodin that is produced within the leaves, bark, and unripe green fruit. Emodin has been found to deter insects and herbivores from eating the bark and fruit, preventing early consumption, and furthering its invasive success. Emodin is also capable of inhibiting native species that are surrounding buckthorn, via leachates in the soil that can also leach into nearby water (Heimpel et al., 2010). Multiple studies on amphibians have shown that emodin is capable of increasing embryo mortality, stunting growth, and increased malformations, in three species of frogs: the northern leopard frog (*Lithobates pipiens*), western chorus frog (*Pseudacris triseriata*) that reside in their invaded range, and African clawed frog (*Xenopus laevis*) from buckthorn's native habitat (Curtis & Bidart, 2021; Sacerdote & King, 2014).

Earthworm Facilitation

Buckthorn has been identified as facilitator of many invasive species. According to Heimpel et al. (2010) buckthorn also furthers potential invasional meltdown by facilitating common earthworm invasion, which can in turn facilitate invasive Asian predatory flatworm (*Bipalium adventitium*). *B. adventitium* prey specifically on common earthworms, suggesting that the invasion of this predatory species was due to increases in earthworm populations. Invasion by common earthworms can be traced back before the introduction of buckthorn, and while studies have been done that show the earthworms without buckthorn, all studies of buckthorn that involve soil biodiversity also include common earthworms.

Historically, the Midwest was without native earthworms, but *L. terrestris* now can be found throughout the Midwest. The biomass studies show that common earthworms are more abundant in areas dominated by buckthorn. This supports the study findings that these earthworms tend to prefer the higher-nitrogen buckthorn litter over oak leaf litter. The increases in earthworms in dense buckthorn stands help decrease the litter layer, which increases bare spots in the soil, and allows for more germination of new buckthorn stems. Invasion by common earthworms facilitated by buckthorn has been challenged by Wyckoff et al. (2014) and Ziter and Turner (2018). As previously mentioned, earthworm invasion occurred before buckthorn invasion in the history of North America. There is a strong suggestion to further investigate the relationships between these two highly invasive and prevalent species (Heimpel et al., 2010; Heneghan et al., 2004, 2006; Knight et al., 2006; Schuster et al., 2021; Wyckoff et al., 2014).

Soybean Aphid and Oat Crown Rust Facilitation

The next species buckthorn assists in facilitation of is the soybean aphid. Invasive soybean aphids are able to cause 40% or more damage to soybean crops in a single year. During the summer months, the aphids reproduce and feed on soybean crops. The changing of seasons and shortening of days trigger instinctual migration to surrounding buckthorn stands where they continue to reproduce into the autumn. The eggs produced are capable of surviving overwintering on their hosts. Once spring temperatures begin to rise and soybeans begin to grow, soybean aphids migrate back to the soybean fields and continue to reproduce until the cycle continues again in the autumn. (Heimpel et al., 2010; Lagos-Kutz & Hartman, 2021)

Oat crown rust (*Puccinia coronata*), like soybean aphids, overwinters on buckthorn. Oat crown rust is a disease capable of causing crop damage and financial strain for producers. A major concern with increasing buckthorn populations is that it will allow for a widespread host

distribution that could facilitate more virulent strains of this disease, and more easily transport the disease across invaded areas. The same concern ties back into soybean aphids; increases in host distribution will allow for a larger and further reaching invasion of aphids that facilitate larger and farther-reaching invasions of Asian ladybeetles (Heimpel et al., 2010).

Mammal Interactions

Buckthorn's affinity for the temperate woodlands of North America exposes our native wildlife to the harmful results of a successful invasion. White-tailed deer (*Odocoileus virginianus)* are often found in areas where buckthorn are not present. The chemical emodin is likely responsible for this trend, as emodin can cause diarrhea, vomiting, and dehydration. Thus, white-tailed deer will not consume the leaves or the bark of the tree and seem to avoid buckthorn stands when feasible (Vernon et al., 2014). This allelopathic tendency is another example of how buckthorn tends to drive away native species. Another potential cause for this trend is the altered understory biodiversity coupled with emodin laden trees. There is still a need for more research on interactions between buckthorn and white-tailed deer, emodin, and the differences in population distribution as a result of these interactions.

As previously mentioned, American robins will nest in buckthorn stands but experience higher predation by mesocarnivores. Coyote (*Canis latrans),* Virginia opossum (*Didelphis virginiana),* and raccoon (*Procyon lotor)* have all been found present in buckthorn invaded habitat. Raccoons are found more often in buckthorn stands in autumn, while many other mesocarnivores have a strong presence in the spring. Invaded stands also experience small mammal abundances, which increase seed predation dynamics within the stands (Bartowitz & Orrock, 2016; Vernon et al., 2014). The previously mentioned rodent interactions along with mesocarnivore data suggest a potential shift in predator prey dynamics in terrestrial woodlands

where buckthorn facilitates increases in rodent populations and seed predation, thus facilitating an increase in mesocarnivore interactions with the invaded stands. The potential for buckthorn to interact with the number of species mentioned continues to reinforce the need for more research into these specific biological interactions and their potential effect on the ecology of temperate woodlands.

Ground-Dwelling Insect Interactions

Emerald ash borer-induced mortality of *Fraxinus spp.* is one example of an insect interaction that results in buckthorn facilitation. Buckthorn has a negative effect on the abundance of ground-dwelling insects, as well as the taxonomic richness of ground-dwelling insects that would normally be inhabiting those areas, were it not for the buckthorn invasion. A comprehensive study on buckthorn revealed that 40 species of ground beetle and 24 species of ants were found to be less prevalent in buckthorn invaded environments (Schuh & Larsen, 2015). Taking allelopathy into consideration, emodin is known to deter insects. Emodin leachates in the soil would be strong enough to deter many native insects from the soil underneath invaded stands (Iannone et al., 2015; Schuh & Larsen, 2015). Like the other species relationships mentioned, there is a need for more research on these interspecies interactions, their causes and effects, and how to combat a multi-species invasion.

Chemical Removal Methods

Buckthorn removal has been proven to allow native species to regenerate at rates that depend on the level of invasion. The ability for buckthorn to invade a deciduous stand to the point where soil composition alterations are able to deter native flora from regenerating at a normal rate furthers the need to act quickly and effectively in the removal of invaded buckthorn stands. Both chemical and non-chemical removal methods require multiple treatments, vigilant

surveying and scouting, consistent marking, and consistent removal methods to ensure effective treatment. It can take 10 or more years to fully eliminate buckthorn stands. The highly invaded stands take significant amounts of time, resources, and labor to ensure proper restoration of invaded areas. The six chemical removal methods discussed are Roundup™, Tordon RTU ™, Brushmaster ™, Garlon 4™, Stalker ™, and Fosamine ammonium (Table 1). Non-chemical removal methods include cutting and girdling, mowing, burning, and Buckthorn Baggies. These removal methods vary in their environmental effects, effectiveness, and costs for products, equipment, and labor.

Table 3

Chemical Buckthorn Removal Methods

Herbicide	Active ingredients	Dilutant	Dilution
Roundup	Glyphosate: N-(phosphonomethyl)glycine	None	100%
Tordon RTU	Picloram: 4-amino-3,5,6-trichloropicolinic acid, triisopropanolamine salt	None	100%
Brushmaster	2,4-D, 2-ethylhexyl ester: 2,4 dichlorophenoxyacetic acid 2,4-DP-p, 2-ethylhexyl ester: (+)-R-2-(2,4-dichlorophenoxy) propionic acid Dicamba: 3,6-dichloro-o-anisic acid	Diesel Oil	8%
Garlon 4	Triclopyr: 3,5,6-trichloro-2 pyridinyloxyacetic acid, butoxyethyl	Diesel Oil	33%
Stalker	Isopropylamine salt of imazapyr	Diesel Oil	8%
Fosamine ammonium	Fosamine ammonium	Water	14%

Roundup

Roundup is the most commonly used herbicide on a global basis. Roundup is currently used in recreational practices, residential areas, and agriculture industries. The active ingredient

glyphosate is a compound that prevents plants from being able to produce important amino acids and transfer nutrients up to the leaves of the plant, killing the plant in a short amount of time. Application of Roundup is only for stumps or stems that have been cut. A basal bark application is not a recommended treatment plan for buckthorn with Roundup. Glyphosate readily bonds to soil, which can prevent root uptake by neighboring plants, however, this creates the potential for heavy rains to wash contaminated soil into other areas and into nearby waterways. Glyphosate is one of the most studied herbicides, and there is plenty of data that details the harmful environmental implications of glyphosate use (Delanoy & Archibold, 2006; National Center for Biotechnology Information, 2022a; Pergams & Norton, 2006).

The study by Delanoy and Archibold (2006) is heavily discussed, as it is one of the most comprehensive articles on the treatment of buckthorn with multiple chemicals to test their effectiveness and determine their cost breakdown. Roundup is the most effective of the three most effective chemical treatments along with Tordon RTU and Stalker. According to Delanoy and Archibold (2006), Roundup was detectable in 36% of the 154 water samples that were taken from the Midwestern U.S., which is likely attributed to the mentioned soil interaction properties of glyphosate. Additionally, water contamination has been directly linked to increased mortality and decreased embryonic growth in amphibians (Corace et al., 2008; Pergams & Norton, 2006). When a 50% solution of glyphosate is used, there is a 95% effectiveness with stump application on stumps that have been cut or girdled. Treatment of stumps that were uncut or girdled stumps is less effective. This study estimated costs at $0.50/oz of treatment. The costs given did not include labor and time for removal (Au & Tuchscherer, 2014; Delanoy & Archibold, 2006; Nagel et al., 2008; National Center for Biotechnology Information, 2022a).

Tordon RTU

Tordon RTU was found to be more effective than Garlon 4 or Bushmaster, but less effective than Roundup and Stalker applications. Similar to Roundup, Tordon RTU must only be applied to cut or girdled stumps, not basal bark application, and also readily binds to soil with a high risk of runoff contamination. The benefit of Tordon RTU is that it tends to be less harmful to mammals and aquatic organisms, but still poses great risk of pollution and contamination in large quantities. Tordon treatments are able to remain in the soil for 29 days to 3 years, with an average half-life of 201 days. While this treatment method poses less risk for organism harm, its lengthy stay in the soil can be detrimental to the soil health of the surrounding treated areas. Tordon RTU is the second cheapest treatment at $0.28/oz (National Center for Biotechnology Information, 2022b; Pergams & Norton, 2006).

Brushmaster

Brushmaster has three active ingredients that range in characteristics. Isooctyl 2,4-D esters is the first active ingredient with a threat combustion if exposed to too high of a heat source or if diluted with diesel oil. This compound can also result in negative effects on small rodents in the form of cancer and poor fetal development (National Center for Biotechnology Information, 2022c).

Dichlorprop-p-2-ethylhexyl is the second active ingredient and has been documented to increased fetal abnormalities in mice, and can cause kidney function decline, musculoskeletal abnormalities, muscle spasms, coma, and death in mice and rats. This compound is harmful to waterfowl, fish, crustaceans, and aquatic algae (National Center for Biotechnology Information, 2022d). Dicamba is a common herbicide ingredient and is the third active ingredient in

Brushmaster. Dicamba is not water safe as it can alter liver function and endocrine function in fish (National Center for Biotechnology Information, 2022e).

The following can all be results of exposure to the chemicals within Brushmaster. Brushmaster is a growth regulating herbicide that assists in broadleaf weed control. In addition to being toxic to wildlife, it is also slightly to moderately toxic to humans, depending on the amount and method of exposure. Side effects in humans can range from loss of appetite, vomiting, shortness of breath, and even central nervous system effects. A large drawback of Brushmaster is that it is often diluted with a light mineral oil or diesel oil. The purpose of this is to allow applications in temperatures ranging down to –15 degrees Celsius. The dilution aspect of this chemical, the active ingredients themselves, and the effects of histological lesions, plasma vitellogenin increases, changes in reproduction hormones, and alterations of hormone related gene expression in aquatic organisms make it a treatment method that should never be applied near waterways or wetlands (National Center for Biotechnology Information, 2022e; Pergams & Norton, 2006). There was no cost data within the studies for Brushmaster.

Garlon 4

Garlon 4 is another commonly used buckthorn chemical removal method that is successful with basal bark application and when sprayed on a cut stump during the growing season, unlike to the previous two chemical herbicides. The active ingredient in Garlon 4 can sit in dying plants for three months; degrades and dissolves rapidly in water; is slightly toxic to waterfowl and other birds; highly toxic to fish, aquatic plants, and invertebrates; and should not be used along waterways, wetland, or other sensitive habitats. Another reason for not using Garlon 4 near sensitive areas is that it is often diluted with diesel oil at a 1L:3L ratio of Garlon 4 to diesel oil. Costs can range depending on quantity purchased, and average $0.34/oz of product

(DiAllesandro, 2012; National Center for Biotechnology Information, 2022f; Pergams & Norton, 2006).

The large study done by Delanoy and Archibold (2006) treated 283,362 stems from 2001-2005 via basal bark application at a rate of 62-254 stems per hour, depending on the experience of the crew. Issues also include applications that occur during months when frost is prevalent; the spray mixes with the water and prevents it from being absorbed and can settle in the soil (Delanoy & Archibold, 2006).

Stalker

Stalker is another chemical that can be used in buckthorn removal, and like many of the options above, is best applied using either a basal bark or cut and spray method. There is supplemental data on the effects on aquatic life, but also readily moves through soil, so water contamination can also be expected. The half-life of Stalker varies between land and water. In soil, the half-life can range from 17-63 days, while in water, it is just 48 hours due to being very water soluble. Stalker is second to glyphosate in effectiveness and cheapest in cost at $0.21/oz of product. (National Center for Biotechnology Information, 2022g; Ferrell & Sellers, 2017; Pergams & Norton, 2006).

Fosamine Ammonium

Fosamine ammonium, commercially available as Krenite, is a liquid that is often diluted with water and is used in ground sprayers, but most sprayers need to be augmented before using Krenite. Fosamine ammonium has little information available on costs, but can be rather expensive at $1.00/oz, making it more expensive than the previous five chemical removal options. A benefit of fosamine ammonium is that a little amount of chemical product can be diluted and used at an efficient rate, as well as a 95% mortality rate in buckthorn. In addition to

the high success rate, non-target impacts were reduced by 85% depending on the site and time of application. This study shows a reinforced need for performing removal and treatment applications at specific times to ensure a successful removal with minimal effects on surrounding flora and fauna (Schuster et al., 2020).

Non-Chemical Removal Methods

Non-chemical buckthorn removal methods are often overlooked due to their low success rate and inability to kill off an individual. Hand pulling is also an option for small individuals and saplings. Cutting, girdling, mowing, and burning are the current non-chemical buckthorn removal methods. Buckthorn Baggies are a more recently marketed non-chemical removal method that have the potential to be used as an effective buckthorn treatment method.

Cutting, Girdling, and Mowing

Buckthorn chemical removal methods often have an 85-95% success rate. Unfortunately, most non-chemical removal methods are often ineffective when not coupled with chemical applications. Cutting and girdling buckthorn shrubs and mowing forest understory to cut down buckthorn all have the ability to further invasion when not monitored, treated with chemicals, or Buckthorn Baggies after cutting and removal of the shrub. Cutting and girdling methods are typically discussed in unison. Cutting shrubs involves using a chainsaw or brush cutters to cut individual buckthorn shrubs just above the ground. Without any chemical treatment, cutting the shrub will only facilitate new shoots of regrowth off the cut stump. Girdling involved cutting a 5cm wide ring around the circumference of the individual shrub and removing the bark to disrupt the cambium layer of the shrub. This interruption helps to decrease the flow of food and water between the leaves and the rest of the shrub. Mowing has been discussed in one study, and the result of this study was that mowing understories to get rid of buckthorn is highly ineffective, as

it also removed other native plants and shade sources, facilitating further germination and invasion of buckthorn (Anfang et al., 2019; Delanoy & Archibold, 2006; Pergams & Norton, 2006).

Burning

Burning buckthorn has been noted in multiple materials and studies and simply involves prescribed burning practices in buckthorn stands. Repeated treatments are standard with this practice and can be performed every two to three years. (Minnesota Department of Natural Resources, n.d.; Panke & Renz, 2012). Prescribed burning can suppress invasive species and is a common land management practice. The risk of prescribed burning is the potential for the fire to spread and lose control. Resulting fires cause large tree loss and canopy gaps which can facilitate additional buckthorn germination.

Buckthorn Baggies

Buckthorn Baggies are the non-chemical removal method that is going to be the focus of this ongoing study. Buckthorn Baggies are recyclable, reusable plastic bags that come in 8in x 8in, 24in x 18, or 18in x 24in sizes to be used on a wide variety of sizes of buckthorn shrubs. The product was developed in Woodbury, MN, has been used by the Minnesota Department of Natural Resources, and is going to be the testing product that is used in combination with cutting of buckthorn shrubs throughout this study. The procedure for removal involves cutting the shrub and placing a bag over top of the cut stump. The plastic is thick, black, and is capable of preventing sunlight from reaching the cut stump, greatly decreasing survival odds. Another benefit of using this non-chemical method is not having to pull the stumps or use chemicals. The omission of chemical use and stump removal adds a soil conservation aspect to this product that could warrant further research on helping to return invaded stands' soil to preinvasion levels.

Preliminary testing for this experiment shows promising results that include a high efficiency with an equivalent cost to chemical applications. A non-chemical removal method that is able to be used in more sensitive environments is important when considering buckthorn can thrive along riverbanks and lake shores.

Chapter III: Methodology

Study Location

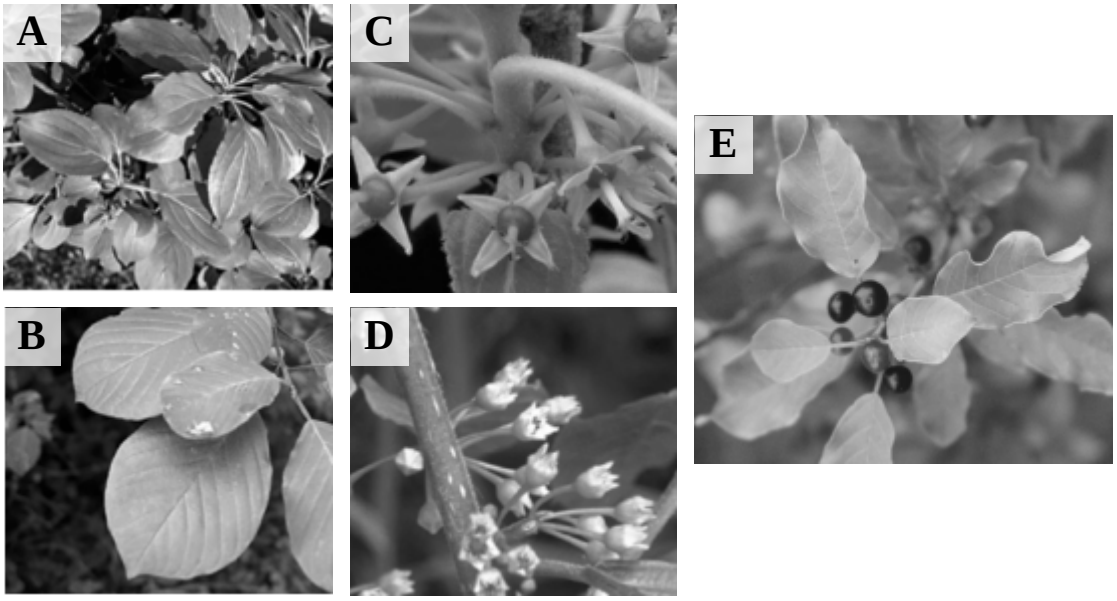
There are two sampling sites being used in this study. The first was located at Sportsman Lake in Owen and Withee, WI. Sportsman Lake is an artificial lake that helps control the water north of Owen to help prevent damaging floods that previously occurred in the town. I was granted permission by Clark County Land Conservation Committee in summer 2021 to remove and bag buckthorn at Sportsman Lake until my research has been completed. The second site was located on a residential lot in Stanley, WI. This residential lot was purchased in February 2021, and in May 2021, a large number of buckthorns were discovered along the property lines.

Field Methods

Buckthorn were identified by examination of leaves, berries, and bark. *R. cathartica* leaves are one to 2.5 inches long, contain three to five pairs of veins that extend towards the tip of the leaf, and have slightly toothed edges (Figure 1 A). *F. alnus* leaves are two to three inches long, containing eight to nine pairs of veins that extend to the smooth edges of the leaf (Figure 1 B). *F. alnus* leaves also have a glossy sheen that is not seen on *R. cathartica*. The flowers of *R. cathartica* are a light green with four petals and the flowers of *F. alnus* are a cream or white color with five petals (Figure 1 C, D). The berries start off as green and fade into red before they turn black in both species (Figure 1 E).

Figure 1

Leaves, Flowers, and Fruit of Buckthorn



Note. Images A and C show European buckthorn (*Rhamnus cathartica*), and images B, D, and E are of glossy buckthorn (*Frangula alnus*). Photos from Minnesota Department of Natural Resources and Panke and Renz (2012).

Even after leaves and berries have been shed for the season, buckthorn can be identified by examination of its bark. Buckthorn bark is a gray brown with light lenticels. Underneath the bark is a dark orange color that makes it easily discernible from other species. While surveying, I took a knife to cut a small piece of the bark to verify it was buckthorn before removal. To ensure verification, two smartphone applications were also used to identify the species before removal. LeafSnap was created by botanist John Kress at the Smithsonian Institute. Seek by iNaturalist is partnered with National Geographic. Both applications compare user-submitted photos of a plant's flowers, berries, bark, and/or leaves to imagery databases to predict the species identification.

Removal took place on August 21, 2021, in Stanley and on August 30, 2021, at Sportsman Lake. I cut each buckthorn trunk 16 cm above the ground. The remaining stump was then covered with a Buckthorn Baggie secured with a zip tie placed tightly around the bag and stump. After I placed the Buckthorn Baggie, I pulled the bottom of the Buckthorn Baggie tightly to the ground, making sure no light could get in.

After bag placement, random bags were chosen for bag checks once a month. The zip ties were cut, and the bags were removed to check for signs of decay, such as fungal growth and the presence of woodlice, termites, ants, and other small arthropods required for the decay process. There were instances where regrowth was spotted coming out of the bottom of the Buckthorn Baggie. When this occurred, I removed the regrowth and replaced the bag to continue the treatment process.

I removed the bags from all stumps on March 26, 2021. Upon removal, a photo was taken to help determine survival. The stumps were left uncovered for two weeks to see if any regrowth occurred and assist in determining the survival of the bagged buckthorn individual. If any new regrowth was spotted, the stump was deemed alive, and I placed another Buckthorn Baggie to continue the treatment. If the stump had no regrowth, and appeared to have minimal to no natural color, it was deemed dead and left uncovered.

Survival Analysis

The survival of buckthorn was modeled using a logistic regression model in program R (R Core Team, 2020). The model included site and stem width as explanatory variables. This analysis aims to verify if the effectiveness of Buckthorn Baggies is related to the size of a buckthorn. The model could also be modified in the future for additional survival estimation based on other factors, like proximity to water or bag placement style.

Cost Analysis

The cost analysis was performed for all six chemical methods and also includes the price/stem removed for Buckthorn Baggies. The cost analysis is based on the number of individuals removed in this study, hours for removal, an hourly pay rate of $15.00, and the cost for one unit of chemical product. Due to a lack of uniform information on costs for buckthorn removal projects that involve the number of people removing the trees, the chemical product costs, and the labor hours required for removal projects, the hours spent to remove the buckthorn individuals in this study will be assumed for all six chemical applications, with the addition of one hour for donning proper PPE and having to dilute and mix the chemicals into sprayers for all chemical applications.

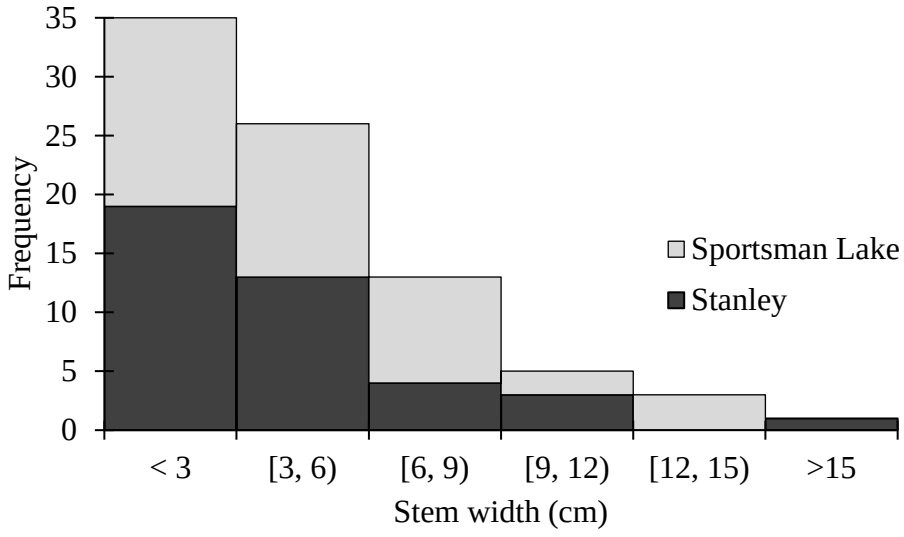
I determined the average cost for the minimum purchasing amount of the 6 chemical methods by taking the first ten prices that are easily accessible through internet searches. The average of the ten prices was taken to determine the average cost for one minimum unit of chemical product. Buckthorn Baggies' cost was determined by selecting the quantity that was required to cover all of the stumps. This included a package of 100 regular 8x8 inch bags and a package of 25 large 18x24 inch bags. Of the 100 regular sized bags, 76 were placed on removed buckthorn stumps, and there were also eight large bags used.

Chapter IV: Results

I cut down 43 buckthorns at Sportsman Lake and 40 buckthorn in Stanley in August 2021 and covered each stump with an appropriately sized Buckthorn Baggie. The average treated buckthorn had a stem width of 4.45 cm (95% CI: 3.64, 5.26), and sizes were similar at the two study locations (Figure 2). I monitored the stumps until March 2022, when I removed the baggies and assessed the survival of the treated buckthorn.

Figure 2

Stem Widths of Treated Buckthorn



Note. Sample sizes were 43 at Sportsman Lake and 40 at Stanley.

Physical Observations

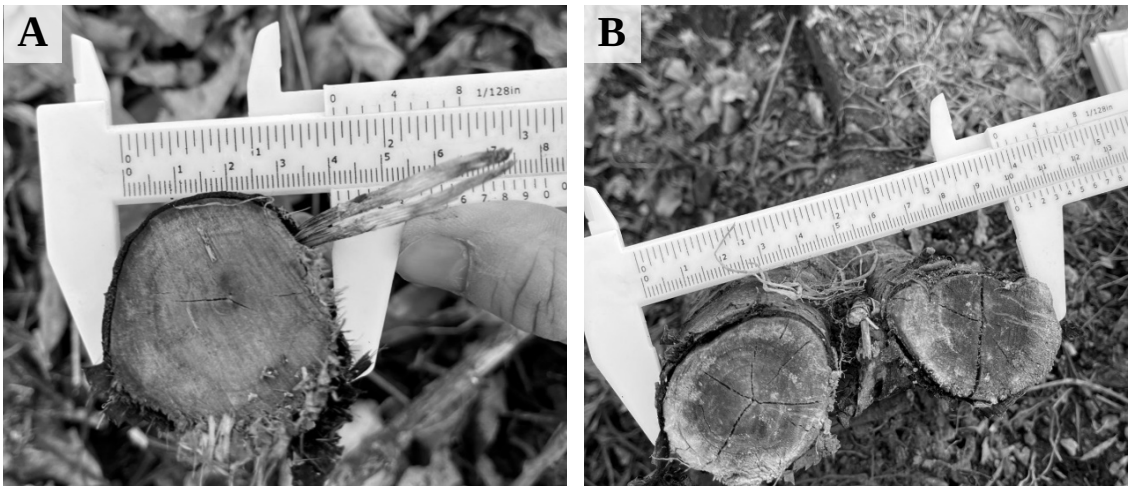
When I removed the Buckthorn Baggies and performed the survival checks two weeks later, I made several physical observations. I determined whether the stump was alive or dead by first looking for loss of the orange interior color of the wood. I noted dead stumps exhibited a dark brown to black bark that split away from the stumps (Figure 3 A, B). The stump wood was also dry and split, indicating a successful kill on an individual (Figure 3 B). I surveyed and

located multiple buckthorns at Sportsman Lake that had large root systems above ground, wider than even the largest Buckthorn Baggie. Stumps that were alive were still very orange two weeks after bag removal (Figure 4 A), and regrowth was visible on two of the larger surviving stumps (Figure 4 B). In instances I was unsure if an individual was alive or dead, I would insert in the stump to find it would disintegrate and fall apart very easily. This often occurred in the stumps with a smaller diameter.

On routine checks for regrowth during treatment, multiple decomposer species were noted underneath the bags, including woodlice and insects (Figure 5 B, C). During the bag removal process, I also noticed the presence of an unidentified white fungus on multiple stumps (Figure 5 A).

Figure 3

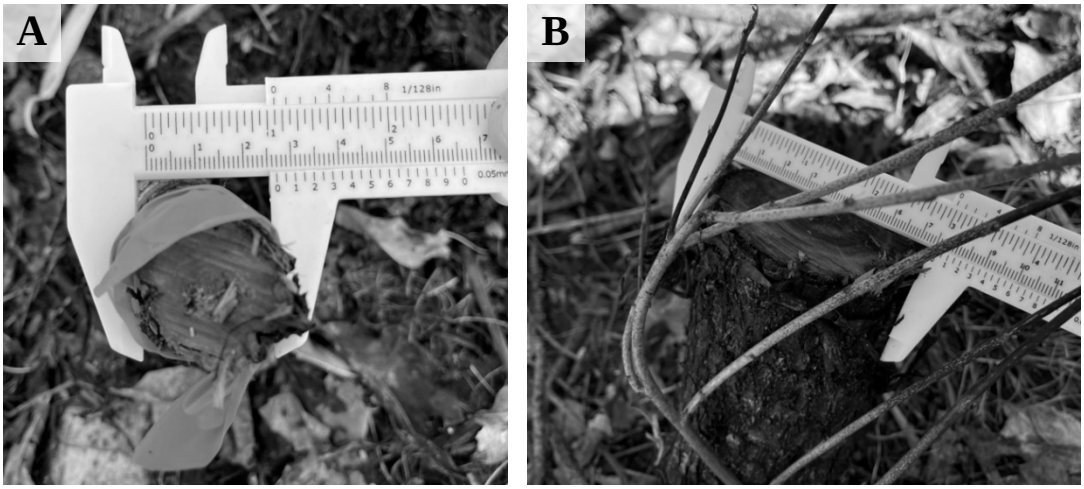
Dead Buckthorn Stumps



Note. Dead stumps may show (A, B) discoloration of wood, bark separation and (B) splitting of the interior wood.

Figure 4

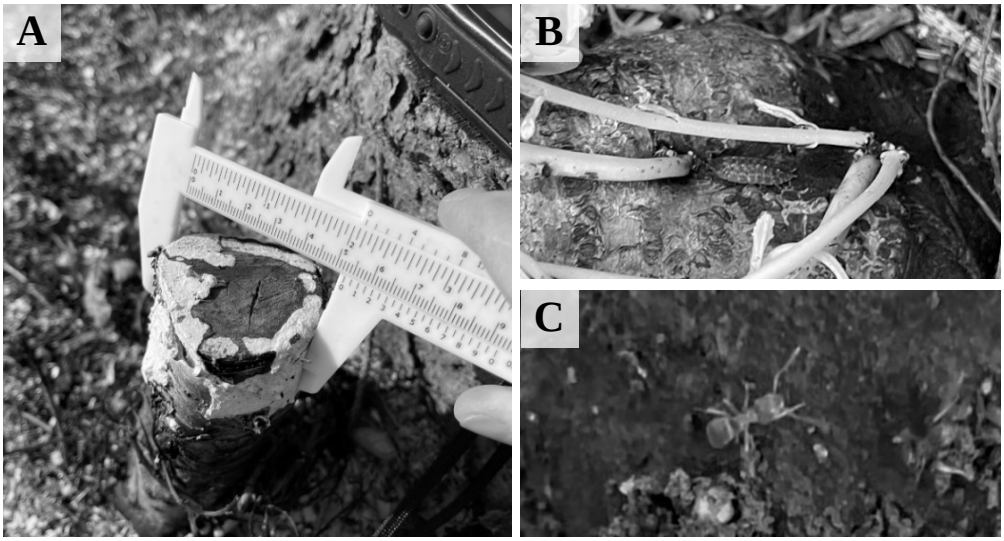
Live Buckthorn Stumps



Note. Live stumps have (A) solid (orange) coloration throughout and often have (B) apparent regrowth after bag removal.

Figure 5

Decomposers Found During Treatment



Note. Decomposers found during treatment included (A) an unidentified white fungus, (B) brickwork woodlice (*Porcellio spinicornis*), and (C) an unidentified ant species.

Survival Analysis

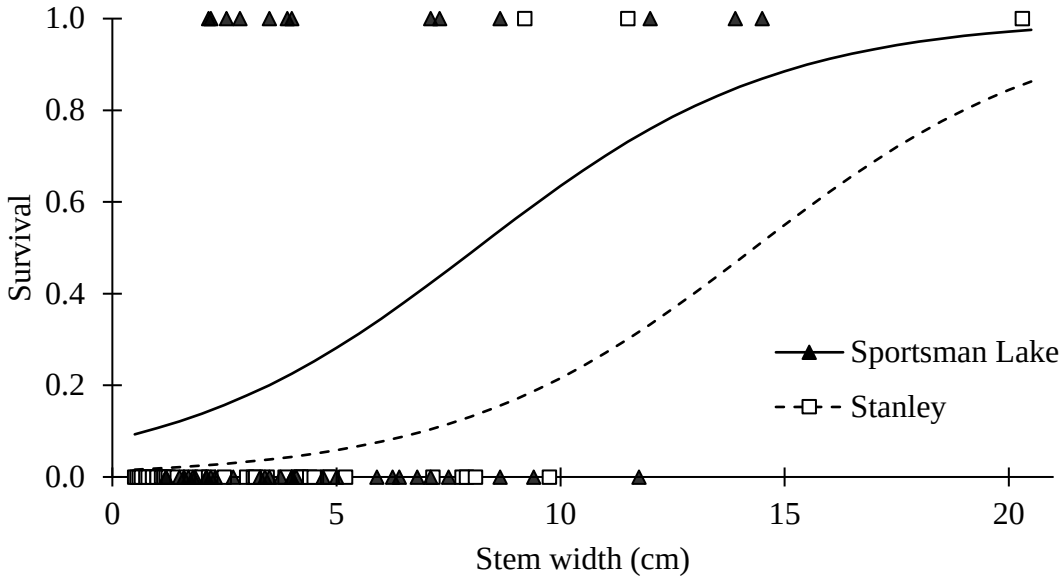
In total, Buckthorn Baggies successfully killed 80.7% (70.8-87.8) of the buckthorn trees over the seven-month treatment period. At Sportsman Lake, 13 of 43 individuals were found alive two weeks after bag removal, meaning the treatment was 69.8% (54.8, 81.4) effective. In Stanley, only 3 individuals were found alive out of the 40 that were treated, for an effectiveness of 92.5% (79.3, 98.0). The logistic regression model was

$$\text{logit}(survival) = -2.426 - 1.844 \cdot site + 0.298 \cdot width,$$

where site = 0 for trees at Sportsman Lake and site = 1 for trees in Stanley. The odds of survival were 84.2% (33.4, 97.5) lower at Stanley than at Sportsman Lake, and they increased by 34.7% (14.6, 65.3) with each 1-cm increase in stem width (z = 3.26, p = 0.001, Figure 6).

Figure 6

Logistic Model of Survival



Note. Size and survival of buckthorn at Sportsman Lake (n=43) and Stanley (n=40) treated with Buckthorn Baggies from August 2021 to March 2022.

Cost Comparison

The cost of Buckthorn Baggies and the six chemical treatment methods depends on the number of individuals being removed. Based on a project of this size, Buckthorn Baggies were the most expensive of the seven removal methods compared (Table 2). Roundup was the cheapest of the seven removal methods and had the lowest average cost when compared to the other chemical treatment methods.

Table 4

Estimated Removal Cost per Stem

Product	Cost per stem (USD)
Buckthorn Baggies	$5.90
Roundup	$4.09
Tordon RTU	$4.13
Garlon 4	$5.02
Brushmaster	$4.84
Stalker	$5.37
Fosamine ammonium	$5.44

Note. Costs were based on removal of 83 buckthorn trees requiring 20 hours of labor at $15.00 per hour. All chemical methods include an additional hour of labor for donning of protective equipment and preparation of the treatment solution.

Chapter V: Discussion

The effectiveness of Buckthorn Baggies differed markedly between the two study locations. There are differences between each site that could be the reason for the statistical difference in survival. The first difference is the site's proximity to water. The majority of individuals alive at Sportsman Lake were close to a water source (Figure 7). This suggests that the proximity to water can affect the survival of an individual with a Buckthorn Baggie. Increased availability of water for the root system could be the reason the stumps closer to the water survived. This could mean there is an additional treatment time requirement for stumps near or within the water line.

The second major difference is land use. Sportsman Lake is a county conservation site, owned and managed on a county level. It is for recreational activity and has walking paths, fishing docks, and forest cover. The Stanley site is a residential property, and the buckthorn were along the property line, likely planted intentionally for decoration. A residential lawn is vastly different in terms of the grasses, weeds, and wildflowers compared to the understory of a forest. The ground cover at Sportsman Lake is a standard deciduous forest with native grasses and forbs. The ground cover at Stanley is minimal and had very little grass or other species growing. Soil chemistry alterations could be a potential reason for the lack of grasses and weeds at Stanley. Buckthorn has been present at Stanley for over 50 years, judging by the largest individual in the study there. Fifty years of excess nitrogen and carbon in the soil has the potential to prevent native species establishing, allowing buckthorn to spread along the entire property line.

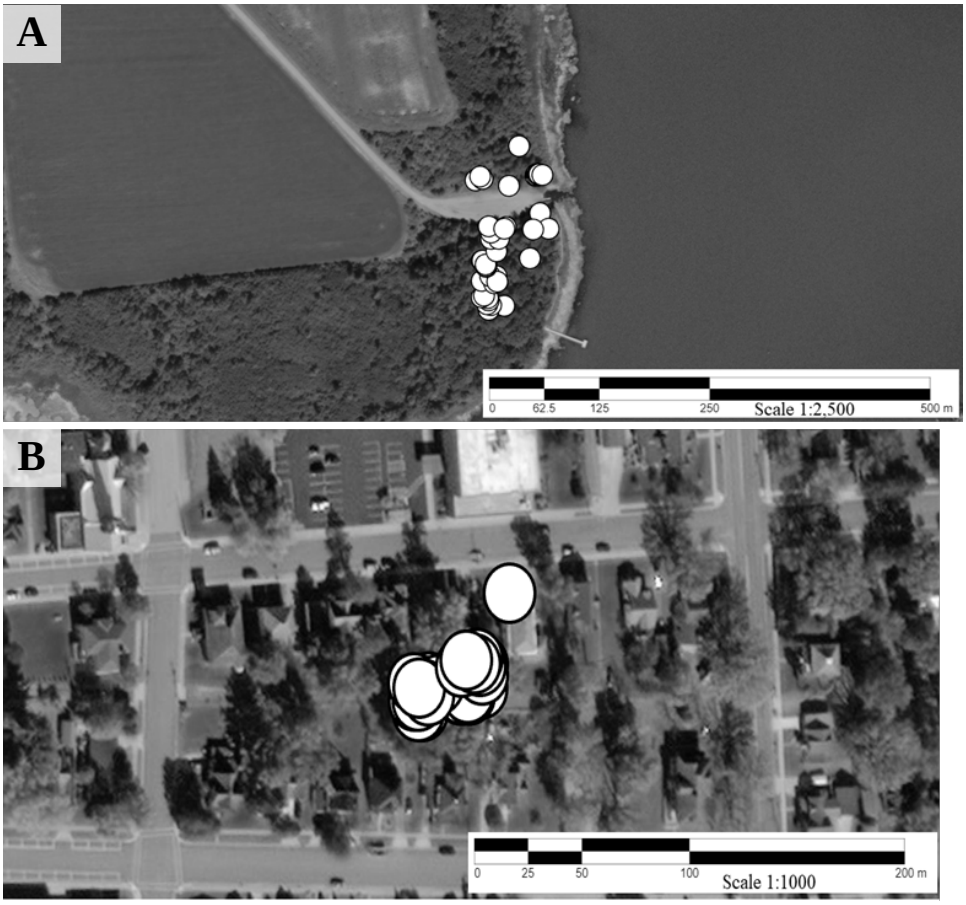
The stem size of buckthorn influences the treatment success of Buckthorn Baggies. The smaller the stem size, the more likely a successful kill after a Buckthorn Baggie placement. The

largest stems between 12 cm and 20 cm were found to still be alive after seven months. After bag removal, there were clear signs of life in the stump. The orange hardwood was still present, and the bark appeared unchanged. The smaller stems were all found to have completely discolored wood, bark split away from the hardwood, and were easy to break or remove from the soil. Knowing the relationship with stem size and Buckthorn Baggie success, it will be beneficial for future studies and buckthorn control and removal operations to know seven months may not be enough time to kill a stem greater than 12 cm.

The 95% success rate of Glyphosate, Tordon RTU, and Stalker are higher than the overall success rate of 80.7% for this study. The success rate at Sportsman Lake of 69.8% is significantly lower than the herbicide success rate and the success rate in Stanley. The Stanley success rate of 92.5% is similar to the chemical application methods and suggests that Buckthorn Baggies are capable of successfully killing invasive buckthorn in areas where chemical applications are not suitable. Chemical treatments are not an option for certain individual buckthorn that may be located in sensitive areas, such as riparian areas. It is important to note that the cost analysis does not take this into consideration, and the higher cost of Buckthorn Baggies may be irrelevant when addressing removal of buckthorn in sensitive areas.

Figure 7

Location of Treated Buckthorn



Note. There were 43 buckthorn treated at (A) Sportsman Lake and 40 treated at (B) Stanley.

Recommendations for Further Research

This study highlights the need of further research on non-chemical buckthorn removal. Buckthorn Baggies have the potential to be a viable alternative to chemical buckthorn removal methods. Based on the findings in this study, it is recommended that Buckthorn Baggies be tested in a more robust study with other treatments for comparison and testing sites that vary in distance between individuals, distance from water, understory vegetation, and canopy cover. A more robust study would provide thorough data on these variables and assist in giving a more well-rounded determination of the relative effectiveness of all seven treatment methods. It was

assumed at the beginning of this study that the success rates for chemicals and the treatment procedures detailed by the manufacturers were properly followed. There is potential that the success rate data for the chemical methods is overestimated by the manufacturers and less than the 95% average gathered from this study. Buckthorn Baggies' success rate was underestimated due to only keeping the bags on the stumps for a seven-month period. Manufacturer's recommendations state a year of placement will yield the best success. Future research should strictly follow the manufacturers recommendations to get a more accurate success rate for Buckthorn Baggies. This research should detail a success rate closer to the chemical treatment methods.

There is also a need for more in-depth studies on all six chemical removal methods and their effects on buckthorn, impact on the surrounding environment, cost, and success rates. Having these data would give a much more comprehensive cost comparison with Buckthorn Baggies. The cost analysis did not take reusability of Buckthorn Baggies into account. If each bag were to be used for two treatments, it would cut the treatment method cost by 50%. This led to an overestimation of the cost per stem removed on Buckthorn Baggies. Repeated studies and taking extra caution during bag checks and removal can ensure reuse of Buckthorn Baggies and lower the removal project cost.

I found the large stumps to be less likely to have been successfully killed in the seven-month bag covering period. This could be due to the large stumps needing more time to die off, or due to a larger root system. More research should also be performed to assess the effectiveness of different Buckthorn Baggie placement methods and durations of treatment. Bags can be placed with the bottom of the bag fanning out on the surface, with the bottom edge folded up to catch rain and help weigh the bag down, or with the bottom edge covered with leaf litter

and duff to help exclude sunlight. A study evaluating bag placement methods and success rates could provide guidance for achieving the best success with Buckthorn Baggies. This study allowed seven months before bags were removed and survival assessed. Additional research should be performed with identical, periodic bag checks to estimate a survivorship curve and predict the time required successful treatment of a buckthorn stump depending on the stem width.

Further research is recommended on the allelopathic tendencies of emodin within buckthorn and its ecosystem. A better understanding of how emodin truly works for and within buckthorn will be critical to understanding the best ways to remove and treat an area after a successful buckthorn invasion. Similarly, additional research on ecosystem relationships between common earthworms, oat crown rust, soybean aphids, and native bird and mammal species with buckthorn is also recommended. Complex relationships between buckthorn and its environment will need to be understood to truly know how an invasion of buckthorn alters the ecosystem in terms of the native flora and fauna that align themselves with buckthorn for a food source or a protection source.

Surveying methods in this study involved using Solocator as a GPS marking tool, I also placed flags at each stump to ensure that each bag was accounted for and easy to track and spot while performing bag checks. Each stump was also numbered and tracked with a spreadsheet to ensure the number of treatments were the same as the bags removed in the final check. Many of the studies reviewed discuss human error in surveying. Often the individual surveying is not the individual spraying, which can lead to missing stumps. Flagging, GPS tracking, and numbering the stumps to accurately depict their location and keep the numbers between surveyed and

treated buckthorn identical. This can be important for checking the stumps each year after removal to guarantee there is no reemergence of buckthorn from that individual site.

There is missing preinvasion data for buckthorn that will continue to be an issue in any study that is performed. Buckthorn has been an invasive species in the Midwest for nearly 200 years, before the impacts of invasive species on ecosystems were really recognized. This lack of preinvasion data will make it difficult for further research on the spreading of buckthorn. Future research is suggested to give a current status of buckthorn invasion in the Midwest or U.S., and truly gauge the extent of its invasion. The lack of robust studies on buckthorn removal methods, both chemical and non-chemical, highlight a need for additional research on forest ecosystems before, during, and after buckthorn invasions. Additional studies that look at the complex ecological relationships between the organisms mentioned in this study would also benefit knowing how to best remove buckthorn.

Conclusion

Buckthorn Baggies are an effective removal method that involves a thick black plastic bag being placed over a freshly cut buckthorn stump. The success rate of buckthorn in this study overall was 80.7%, which is not as high as the reported 95% success rate of glyphosate, Tordon RTU, or Stalker. The other chemical treatment methods' success against buckthorn was unavailable at the time of this study. All treatment methods, including both chemicals and Buckthorn Baggies, require yearly re-checking of the invaded land and multiple treatment applications. The overall success rate of Buckthorn Baggies gives hope for the successful use of a non-chemical application. This can encourage the removal of buckthorn by aquatic locations that previously may not have been considered, due to chemical control methods inability to be used by aquatic areas.

Future research and studies on buckthorn removal methods will need to focus on the individuals' distance from a water body. Additional research on the complex interspecies relationships of buckthorn and other species will assist in knowing how to best remove buckthorn and treat the area during and after an invasion. Emodin leaching is known to have environmental effects, and additional studies on emodin leaching paths through the soil and into waterways can help prevent the uptake and dispersal of emodin during removal. Future Buckthorn Baggie research will need to address bag placement options, length of the placement interval, and proximity to water effecting the survival of buckthorn to ensure the proper placement and best chances of a successful kill.

Consideration needs to be given to the surrounding ecosystem when looking for a treatment/removal method for buckthorn. Currently, there are no chemical treatment methods that are approved for application on or near bodies of water or places where runoff into groundwater is common. Additionally, the six chemical treatment methods all have negative effects on native plants in the surrounding areas. Buckthorn Baggies negate the caution needed when applying herbicides during a removal and avoid the harmful ecological aspects of buckthorn removal. Chemical applications are becoming more controversial as sustainability and environmental friendliness continue to rise in popularity. Buckthorn Baggies are a non-chemical invasive species removal method that could potentially be used to help remove other invasive species without harming sensitive ecosystems.

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