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Assessment of invasive *Gypsophila paniculata* Control Methods in the northwest Michigan dunes

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Assessment of invasive *Gypsophila paniculata* Control Methods in the northwest Michigan
dunes

Emma K. Rice

A Thesis Submitted to the Graduate Faculty of

GRAND VALLEY STATE UNIVERSITY

In

Partial Fulfillment of the Requirements

For the Degree of

Master of Science in Biology

Department of Biology

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Dedication

To my father, for instilling in me a sense of wonder for the natural world and love of scientific inquiry, and to my mother for her steadfast support and willingness to proofread endless versions of this thesis.

Acknowledgements

I would like to thank all of the individuals that made this thesis possible. First, I would like to thank The Nature Conservancy and National Park Service for partnering with us on this project, their removal crews for the incredible work in managing *G. paniculata* and willingness to coordinate treatment timing and location. I thank my graduate advisor, Dr. James McNair, for his guidance, support and enthusiasm throughout this project. I would also like to thank my co-principal investigator and graduate committee member, Dr. Charlyn Partridge for the additional support, advice and aid with field work. My other graduate committee members, Dr. Timothy Evans and Dr. Daniel Frobish, for providing advice and support whenever sought. Thanks to Hailee Leimbach-Maus for being an incredible baby's breath co-researcher and never hesitating to lend a helping hand. I thank Kurt Thompson, Kim Oldenborg, Matt Kienitz, Emily Dusicska, Alexis Hoskins and Pamela Martínez-Oquendo for their assistance with field work and mapping for this study. Funding for this research was provided by the U.S. EPA Great Lakes Restoration Initiative to Charlyn Partridge, James McNair, and Kevin Strychar. Additional funding was provided by the Presidential Research Grant through Grand Valley State University.

Abstract

Gypsophila paniculata is an invasive species in Michigan's northern lower peninsula and a problem invasive in much of the northern United States and Canada. *Gypsophila paniculata* readily outcompetes native plants in sandy, well-drained soils due to its deep taproot, which allows access to scarce resources. It reproduces and disperses mainly by seed, but the phenology of seed maturation is poorly understood. *Gypsophila paniculata* is of particular concern in lakeshore dunes because the areas where it is most dense are also populated by several endemic and threatened species. Despite many years of intensive management, high densities of *G. paniculata* persist in previously treated areas. To determine why this occurs, our research assessed current removal methods (foliar application of glyphosate and manual removal) by (1) measuring *G. paniculata* density and frequency over a large area using a point-intercept grid before and after treatment from 2016-2018, (2) investigating how timing of treatment affects *G. paniculata* density, (3) determining the resprout frequency of treated plants, (4) characterizing the local seed-maturation phenology and (5) investigating how timing of treatment affects resprouting and seed germination. Our results confirm that treatment for one or two years reduces the density of *G. paniculata* but does not extirpate it, overall treatment is most effective from late June through early July, and a small percentage of manually removed plants and herbicide-treated plants resprout following treatment. Seed germinability increased quickly at the end of July and reached a maximum of 90% or higher by early August in both 2016 and 2017. The germinability of seeds from plants sprayed with glyphosate in early, mid, and late July 2016 was higher the later treatment was administered, highlighting the need to treat early in the

growing season. To effectively control *G. paniculata*, we recommend that managers treat for consecutive years to remove regrowth and missed plants and focus treatment from late June to early July for best control, including prevention of seed maturation. Ultimately, this information will contribute to an adaptive management plan specific to baby's breath that can be used in infested areas throughout northern North America.

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Abbreviations

SBDNL – Sleeping Bear Dunes National Lakeshore

TNC – The Nature Conservancy

NPS – National Park Service

Chapter 1

Introduction

Invasive species impacts

Invasive species are defined as non-indigenous species that threaten biological diversity and cause or are likely to cause economic or environmental injury in their introduced range (Beck et al. 2006, CBD 2008). The severity of environmental and economic impacts of invasive species around the world is second only to that of human population growth (Pimentel 2011). The most heavily studied invasive species are those that negatively impact agricultural production, but studies of invasive species and their impacts in nonagricultural areas are rapidly increasing in number. Invasive species are known to cause a large number of negative effects, including reducing diversity and abundance of native organisms, altering natural fire regimes, decreasing food web complexity, altering nutrient cycling and the hydrological cycle, impeding recreation and degrading aesthetics (Elton 1958, Ehrenfeld 2003, Reid et al. 2009). Many of these impacts can ultimately lead to degraded ecosystem function (Reid et al. 2009).

These negative consequences cost countries billions of dollars annually in reduced agricultural production, degradation of natural spaces and concomitant losses of income from tourism and recreation, and reduction of overall biodiversity. In the United States alone, costs associated with environmental disturbance and reduction of biodiversity due to invasive species and associated control programs are estimated to total 120 billion dollars per year (Pimentel et al. 2005). A specific example is purple loosestrife; this single species costs an average of 48 million dollars per year to control (Pimentel et al. 2005). There are approximately 50,000 invasive species in the United States, and the number is continually increasing due to environmental

disturbance from human population growth and increased global travel and trade (Pimentel et al. 2005).

Invasive species that become established pose a great risk to threatened and endangered species today. In the United States, invasions of plants, animals, and microbes are thought to be partly responsible for a 42% decline in native species that are now on the threatened and endangered species list (Pimentel 2011). Ironically, most of the invasive exotics now found in the United States were introduced intentionally for food, as biological control agents for other species, or as garden ornamentals. As a result of these introductions, invasive species are spreading at a rate of about 700,000 hectares a year in United States wildlife areas, having a great impact on national parks and other natural lands (Pimentel et al. 2005).

Adaptive management

Adaptive management is a systematic experimental approach to management of natural resources (Holling 1978, Walters 1986). In marine fisheries management and the management of certain wildlife species (e.g., North American ducks), adaptive management has been implemented rigorously and quantitatively using sophisticated stochastic control models of resource dynamics as altered by specific management policies (Walters 1986). In other applications, such as forest management (Taylor et al. 1997) and managing invasive plants (McNair 2005), a less formal approach is used and the basic concept is to view management as an experiment. Thus, adaptive management involves the use of thoughtful experimental design with replication, the use of quantitative assessment measures that are reliable indicators of the degree of success of each treatment (such as changes in densities of invasive species and native plants), the recording of these assessment measures before and after administering management

treatments, and rigorous statistical methods to objectively determine the strength of evidence for the efficacy of a given treatment or the relative efficacies of alternative treatments. Monitoring and rigorous analysis of measurable assessment indicators make it possible to optimize management for a particular area of study by comparing treatment outcomes. In order to manage an ecosystem properly, one must also understand the structure and dynamics of that system to a reasonable degree (Holling 1978) and treat the process of management as a continuous experiment (Walters 1986, Berkes et al. 2000). Moreover, once a successful control method is determined, it is not set in stone; as the system changes, the best management practices also must adapt, requiring periodic reassessment (Walters and Hilborn 1978). While this method is expensive and time consuming (Walters 1986, Lee 1999), adaptive management is thought to be a more effective alternative to the traditional management practice of using professional judgment to guess which treatment method will be most effective and then implementing that one control measure, with no objective or rigorous method for assessing the outcome.

Methods for invasive species control

A variety of treatment methods have been used to control invasive species. These methods can be divided into three main categories: biological, mechanical and chemical. Biological controls commonly include introducing predators, parasites and pathogens that feed on invasive plants in their native range and have co-evolved as natural enemies (Messing and Wright 2006). Care must be taken when considering using biological control, because the introduced control species might negatively impact non-target native taxa and potentially become a new invasive species itself, as has happened many times in the past (Messing and

Wright 2006). For this reason, introduction of alien biological control species is tightly regulated by the federal government.

Mechanical control techniques are numerous and often are used as a cost-effective method in small-scale, low-density invasion areas. Examples of mechanical control methods include cutting (mowing, weed whacking, string trimming, chainsaw, root severing), hand pulling, grazing, tilling, litter removal, burning, mulching, water level manipulation, soil amendment and native revegetation (Kettenring and Adams 2011). Several of these methods can be used by volunteers with minimal training.

The most commonly used method of control for large-scale invasions is chemical control using herbicide (Kettenring and Adams 2011). Contact herbicides, as the name implies, kill the part of the plant that has come in contact with the herbicide. Systemic herbicides, on the other hand, are absorbed by the roots or foliage and translocated throughout the plant, leading to injury or mortality. Selective herbicides kill or harm certain types of plants without substantial harm to others (e.g., 2,4-D selectively kills dicotyledons like dandelions but does not kill monocotyledons like grasses), while nonselective herbicides (e.g., glyphosate) kill or harm all plants in the chemical application area (Lingenfelter 2013). To be effective at killing the target invasive species, a systemic herbicide needs to have sufficient contact with and be absorbed by the plant, move within the plant to the site of action without being deactivated and accumulate to toxic levels at the site of action (Lingenfelter and Curran 2013). Herbicide can be applied in several ways, the most popular of which are spot treatments (common in natural areas) and broadcast spraying over a large area (more common in agriculture). The herbicide that is most popular in the U.S., due to its relatively low cost and nonselective properties, is glyphosate

(systemic, nonselective), followed by 2,4-D (systemic, selective), picloram (systemic, selective), and triclopyr (systemic, selective) (Kettenring and Adams 2011).

***Gypsophila paniculata* in northwest Michigan**

Gypsophila paniculata (baby's breath) is the invasive species of focus for this thesis.

Gypsophila paniculata is an herbaceous perennial native to the Black Sea region of Eurasia. It was introduced to North America in the late 1800s as a garden ornamental in Manitoba, Canada (Darwent and Coupland 1966) and was first documented in Michigan in 1913 in Emmet County, at the northern tip of the lower peninsula (Gleason s.n. MICH). In the area around Sleeping Bear Dunes National Lakeshore (northwestern lower peninsula), *G. paniculata* was first observed at Point Betsie in the 1950s (Kleitch 2009). Since that time, it is thought that *G. paniculata* spread predominantly to the northeast along 260 kilometers of Lake Michigan's shoreline.

Certain life history stages of invasive species have been identified that are associated with prolific invasion. Some of the indicators for high-risk invaders, especially agricultural invaders, include prolific reproduction (vegetative or sexual), abiotic dispersal and minimal pre-germination seed requirements (such as lack of dormancy and need for scarification; Sakai et al. 2001). *Gypsophila paniculata* expresses all of these life history characteristics. Also aiding in its success in the northwest sand dunes of Michigan is its preference for dry, sandy, alkaline, and calcium-rich soil, allowing it to become one of the most aggressive and destructive invasive non-indigenous plants threatening the region today (TNC 2013). For that reason, *G. paniculata* is a priority invasive species for detection and control in Michigan's northern lower peninsula (DNR 2015).

Purpose

The purpose of this study is to assess the effectiveness of the current *G. paniculata* control methods used in northwest Michigan. The same methods have been used for many years in our research sites (mechanical removal 28 yrs and herbicide 11 yrs), but their combined effectiveness has never been rigorously assessed. This research aims to partially fill that gap.

Scope

This research is focused on *G. paniculata* in northwest Michigan and on the two main treatment methods used by managers to control it (manual removal and foliar application of glyphosate). However, the findings from this research can be applied to other states and provinces where *G. paniculata* is invasive.

Assumptions

When designing this study, I assumed that the treatment provided by different sets of crew members in the two summers spanned by the study would be similarly applied. I also assumed that the reference site I chose, after looking at the topography and vegetation cover, would provide an acceptable indication of what likely would happen in the treatment sites if they were not treated, making it reasonable to attribute statistically significant changes detected in the treated areas but not in the reference area to the treatment instead of weather or other factors that could not be controlled.

Research Questions

This master's thesis project is intended to contribute to a longer-term goal of creating an adaptive management plan, specific to the invasive plant *G. paniculata*, that can be used in areas of North America and Canada where infestations occur. To create this management plan, the effectiveness of methods currently employed by managers, as described above, must be assessed. These methods were chosen for use by The Nature Conservancy and Sleeping Bear Dunes National Lakeshore staff on the basis of unpublished preliminary assessments, but important questions remain, including:

1. How effective are manual removal and herbicide application in reducing the frequency and density of *G. paniculata* in treated areas?
2. When is the best time to employ each control method?
3. How effective are manual and herbicide treatment methods in preventing resprouting of treated plants?
4. What is the phenology of seed maturation in coastal dune habitats of northwest Michigan?
5. How is germinability affected by the timing of glyphosate application?

All of these questions are addressed by this Master's research project.

Significance

This research generates new empirical data to inform future management of invasive *G. paniculata*. In particular, it provides the first rigorous assessment of the phenology of *G. paniculata* seed germination in natural lands of North America, the first rigorous assessment of how the efficacy of glyphosate in preventing production of viable seeds depends on the timing of

application, the first rigorous assessment of the efficacy of manual removal and foliar application of glyphosate in actually killing treated plants (so that resprouting does not occur), and the first rigorous assessment of the efficacy of standard management practices (a combination of manual removal and foliar application of glyphosate) as implemented by typical field crews in reducing the abundance of *G. paniculata*. When the findings of this research project are incorporated into an adaptive management plan specific to *G. paniculata*, The Nature Conservancy and the National Park Service will be able to more effectively control infestations while optimizing their resources. More broadly, *G. paniculata* is invasive not only in Michigan but in all northern states in the United States and much of Canada. The management plan to which this project will contribute can be adapted by managers in these other areas, and can also be adapted to other invasive species in northwest Michigan dune habitats, such as *Silene vulgaris* (bladder campion). The ultimate result based on implementation of this research will be improved control of invasive *G. paniculata* and protection of native species.

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Chapter 2

Assessment of Invasive *Gypsophila paniculata* Control Methods in the Northwest Michigan Dunes

Abstract

Gypsophila paniculata is an invasive species in Michigan's northern lower peninsula and is a problem invasive in much of the northern United States and southern Canada. *Gypsophila paniculata* readily outcompetes native plants in sandy, well-drained soils due in part to its deep taproot, which allows access to scarce resources. *Gypsophila paniculata* is of particular concern in lakeshore dunes because the areas where it is most dense are also populated by several endemic and threatened species. Current removal methods include foliar application of glyphosate and manual removal. Despite many years of intensive management, high densities of *G. paniculata* persist in previously treated areas. To determine why this occurs our research assessed current removal methods by measuring *G. paniculata* density and frequency over a large area using a point-intercept grid before and after treatment, investigating how timing of treatment affects *G. paniculata* density and determining the resprout frequency of treated plants. Our results showed a statistically significant decrease in density of *G. paniculata* after one and two years of treatment ($p < 0.001$) in all study areas, but mixed results for a decrease in presence-absence frequency ($p = 0.28$, $p = 0.11$, $p < 0.001$). Herbicide and manual treatments were most effective when applied in late June and early July. Resprouting of marked plants within one year of treatment was seen in 14% (7-26%, 95% binomial CI) of the manually removed plants and 2% (0-11%, 95% binomial CI) of the herbicide treated plants. Our results confirm that one and two years of treatment reduces the density of *G. paniculata* but does not extirpate it, that overall

treatment is most effective after late June, and that a small percentage of manually removed plants and herbicide treated plants resprout within the same season and one year following treatment. To effectively control *G. paniculata*, managers should treat for consecutive years to remove regrowth and missed plants and focus treatment from late June to early July for best control, including prevention of seed maturation.

Restoration Recap

- *Gypsophila paniculata* is a problem invasive species in much of northern North America, but little is known about its response to treatment on a large spatial scale, the time of year when treatment is most effective or its resprout potential following treatment.
- Our results show that *G. paniculata* infestations require multiple years of treatment to reduce plant presence and that herbicide is more effective than manual removal in reducing density and resprouting.
- The optimal time for *G. paniculata* treatment in northwest Michigan is from late June to late July.

Introduction

Invasive plants have the potential to reduce the abundance and diversity of native biota, modify ecosystem processes, and have large economic impacts (Reid et al. 2009; Pimentel et al. 2005). For these reasons, removal of invasive plants is often a key initial step in restoring degraded habitats. A substantial amount of resources are used to treat invasive species, so managers and restoration practitioners need to know what treatments are most effective to optimize control.

Gypsophila paniculata (baby's breath) is an herbaceous perennial plant native to Eurasia. Since its introduction to North America in the 1800s as an ornamental plant, *G. paniculata* has become invasive in Canada and the northern United States (Darwent and Coupland 1966). It is highly invasive in coastal dune habitats of northwest Michigan, where it was first documented in Emmet County in 1913 (Kleitch 2009, Reznicek et al. 2011). *Gypsophila paniculata* readily outcompetes native plants in well-drained, sandy soils due to its deep taproot (up to four meters), allowing access to scarce resources (Darwent 1975). *Gypsophila paniculata* produces large numbers of seeds annually (14,000 per plant: Stevens 1957), which are dispersed in tumbleweed fashion over distances of just under a kilometer as wind severs the base of brittle plant stems at the end of the growing season (Darwent 1975). This dispersal mechanism allows plant stems to move through the natural dune topography, spreading seeds and collecting in large numbers in shrubs and forest edges, leading to monocultures of *G. paniculata*.

Dunes are naturally disturbed environments, and for that reason they are highly susceptible to invasion. Sleeping Bear Dunes National Lakeshore (SBDNL), on the northwest coast of Michigan's lower peninsula, is part of the largest freshwater dune system in the world and is an important ecological and economic resource for Michigan (Albert 2000). This dune system is also home to several threatened, endangered and endemic species, including the piping plover (*Charadrius melodus*), Lake Huron locust (*Trimerotropis huroniana*), and Pitcher's thistle (*Cirsium pitcheri*). The foredune and secondary dunes have the highest concentration of threatened and endemic species in the dune system at SBDNL (Albert 2000) and this is where the invasive *G. paniculata* is most prevalent, underscoring its threat to these species (DNR 2015). *Gypsophila paniculata* currently infests roughly 25% of the natural lands at SBDNL and in some areas constitutes over 80% of the vegetative cover (Vandermeulen 2006). A previous

study on dunes in Latvia, where *G. paniculata* is also invasive, found its spread is limited by the presence of stable dune plant communities and promoted by natural and anthropogenic disturbance (Rudzite 2002). However, it is unknown whether *G. paniculata* established and proliferated in SBDNL solely because of the disturbed nature of the dunes and heavy foot traffic in the National Park or if this measure of success was at least partially a result of ideal environmental conditions for this species.

G. paniculata is a priority invasive species for detection and control in Michigan's northern lower peninsula (DNR 2015). It has been actively managed in northwest Michigan since the 1990s and in SBDNL since 2006. Between The Nature Conservancy (TNC), the National Park Service (NPS), and the Grand Traverse Regional Land Conservancy, over 600 ha have been treated. *Gypsophila paniculata* cover has been reduced from 50% to less than 10% in some treated areas (Emery et al. 2013), but about 750 ha remain infested and over 2000 ha are ideal *G. paniculata* habitat in SBDNL (GLEPMT 2006, TNC 2013). Regrowth often occurs following treatment, but the reasons for this regrowth are poorly understood. Therefore, additional information is needed to assess the efficacy of current removal methods in reducing *G. paniculata* abundance in treated areas.

The purpose of this study was to aid in the improvement of *G. paniculata* treatment methods by addressing three research questions: (1) How effective are manual removal and herbicide application in reducing *G. paniculata* frequency and density in treated areas? (2) When is the best time to employ each control method? and (3) How effective are manual and herbicide treatment methods in preventing resprouting of treated *G. paniculata* plants?

Methods

Treatment Methods. Current treatment methods employed by TNC and SBDNL crews (seven to fifteen members) include foliar application of 23.4 ml L⁻¹ aqueous solution of glyphosate (4.5 lbs. a.e. per gallon, Roundup ProMax®, Monsanto), administered at differing rates according to plant density. Glyphosate, a non-selective systemic herbicide, is administered with care at this site to prevent accidental spray-over damaging the surrounding native plant community. Manual removal is used when wind speeds exceed 10 mph, rain or high temperatures occur, crews are treating remote areas or in close proximity to threatened *Cirsium pitcheri* (TNC 2013). Manual removal consists of using a spade to sever the taproot below the caudex (where stems meet the root), which is thought to prevent resprouting (TNC 2013). Cut plants are removed from the soil but left on site. Treatment at SBDNL typically begins in early June and continues until early August, by which time plants have seeded. Treatment for the present study concluded in late July.

Point-Intercept Surveys. Pre- and post-treatment point-intercept surveys were conducted in large unmarked portions of the areas treated by field crews employed by TNC and NPS as a routine part of their summer-long treatment efforts at SBDNL. This is an advantageous method for assessing the efficacy of treatment methods as actually applied by field crews, because crews are administering treatment on a large spatial scale and do not know when they are treating the survey areas. Point-intercept surveys are commonly used to assess treatment efficacy in studies of invasive aquatic plants (Madsen 1999, Conklin and Smith 2005, Mikulyuk et al. 2010, Parks et al. 2016) but are less common in similar studies of invasive terrestrial plants.

Point-intercept surveys were conducted in 2016, 2017, and 2018 to assess the effectiveness of current treatment methods used in Michigan. We established grids (50-m mesh) on two 15-ha treatment areas (Exp1 and Exp2) and a 16-ha untreated reference area with similar densities of *G. paniculata* and other vegetation (Figure 2.1). The sampling grid was generated with ArcGIS software (ESRI™ 10.2) and entered into hand-held GPS receivers (Magellan ProMark3), which field personnel used to navigate to the unmarked sampling points. The reference area enabled comparison with the treated areas; post-treatment changes (if any) detected in treated areas are not attributable to treatment if they also occur in the reference area. We sampled a total of 182 points, of which 122 were treated (56 receiving two years of treatment and 66 one year of treatment) and 60 were reference points. The number of *G. paniculata* plants within a 2-m radius around each sampling point was counted and divided by the area of the sampling disk ($4\pi \approx 12.57 \text{ m}^2$) to yield an estimate of local density. In addition, *G. paniculata* was scored as present at a given sampling point if the number of plants counted was greater than zero and absent otherwise; these presence-absence data were used to assess potential changes in frequency of occurrence. Treatment area Exp1 was established in 2016 and surveyed in 2016, 2017, and 2018; treatment area Exp2 was established in 2017 and surveyed in 2017 and 2018. The 2017 and 2018 surveys were completed in four days at the end of May. Due to a miscommunication with field personnel, the 2016 survey occurred over a 2-week period in June in Exp1 area and in a single day in August in the reference area.

Efficacy of the treatment methods was assessed using the mid-P McNemar test for matched-pairs presence-absence (frequency) data (Fagerland et al. 2013) and a nonparametric bootstrap test for count (density) data (R Core Team 2017). These tests assume that post-treatment changes (density, presence-absence) at different survey points are independent; we

assessed this assumption with a spatial autocorrelogram based on Moran's *I* and progressive Bonferroni adjustment for multiple comparisons using a modified version of the `correlog` function in R package `ncf` (Bjornstad 2001).

Timing of Treatment. Large 32-m $\times$ 32-m plots were set up throughout the Exp1 area for treatment. There were 48 plots in 24 pairs, with one manual removal and one herbicide plot in each pair so the densities were similar between treatments. We randomly assigned treatment type within each pair. Each plot pair had a minimum of 30-m of space between neighboring plots. Within each larger treatment plot, one 4-m $\times$ 4-m plot was surveyed for *G. paniculata* density. Locations of these smaller plots were determined by tossing the survey pole into the larger plot. Abundance counts of *G. paniculata* were taken between 0-14 days prior to the day of treatment. The number of *G. paniculata* plants within the survey plot was determined and divided by the area (16 m²) to yield an estimate of local density. Treatment was administered from June through July.

Treatment was performed from June 13 to July 27 in 2016. The timing of plot treatment was separated into three groups: early-season (treatment between June 13 and June 23, 2016), mid-season (treatment between June 27 and July 7), and late-season (treatment between July 11 and July 27). Plants, aside from seedlings, are similar in size throughout this time but at different stages in their phenology. Our goal was to assign 8 manual removal plots and 8 herbicide treatment plots to each group, but several weeks of high afternoon winds in June prevented some of the herbicide plots from being treated until July. As a result, early-season treatment consisted of 10 manual removal and 7 herbicide plots, mid-season of 10 manual removal and 6 herbicide plots, and late-season of 4 manual removal and 11 herbicide plots. Plots were resurveyed in June

of 2017 to assess treatment efficacy. We also surveyed 10 4-m $\times$ 4-m plots in the reference area in August of 2016 and June of 2017. We performed a Tukey's HSD test to determine how treatment type and timing jointly influence density change.

Resprout Following Treatment. To assess resprouting of individual plants following treatment, prior to the 2017 plant removal period, we marked 125 plants that had a minimum of three stems with small aluminum tags affixed to 61-cm metal rebar stakes. The minimum stem number aided in the selection of mature plants, which we felt were the ones most likely to resprout following treatment and therefore the most sensitive indicator of potential treatment ineffectiveness. The stake was placed in the ground at the base of each plant. Plants were checked for health one week after marking to be sure that the rebar did not damage the taproot when placed in the ground. Any plant that showed signs of declining health (5 individuals) was unmarked and removed from the experiment and another plant located within 1-m was marked. Of these 125 plants, 50 were removed manually, 50 were treated with herbicide, and 25 were left untreated in the reference area. Treatment type was assigned randomly to plants. All treated plants were sprayed or manually removed during one week in early June 2017 to maximize the potential for resprout during the growing season. Marked plants were placed within 2-m $\times$ 2-m plots which received manual or herbicide treatment for uniformity; the plots served to prevent drift of herbicide onto manually treated plants. For additional protection, the marked, manually removed plants were placed under a protective dome while exterior areas were treated with herbicide. The condition of the root crown or upper taproot of the treated plants was checked in late fall 2017 and spring of 2018 to determine whether any resprouting occurred. Plants were located using a Trimble Geo 7x and metal detector.

We determined the number of plants that did and did not resprout in each treatment group. We analyzed the data using a large-sample test for the difference between two proportions (Hollander et al. 2015, p. 495-500) with the Newcombe Hybrid Score confidence interval to compare (1) removal methods and (2) each removal method to the reference area.

Results

Point-Intercept Survey. There was a statistically significant decrease in density of *G. paniculata* in the Exp1 area after one year of treatment ($p < 0.001$; Figure 2.2) but no significant decrease in frequency of occurrence ($p = 0.31$). The mean change in density was -0.97 plants/m² (-1.4 to -0.56 , 95% CI). After one year of treatment in the Exp2 area, we saw a significant decrease in both density ($p < 0.001$) and frequency ($p < 0.001$), with a mean density change of -1.79 plants/m² (-2.3 to -1.3 , 95% CI). After two years of treatment in the Exp1 area, the same trend emerged that was seen after one year of treatment, with a significant decrease in density ($p < 0.001$) but not in frequency ($p = 0.11$) and a mean density change of -1.75 plants/m² (-2.3 to -1.2 , 95% CI). The Exp2 area was only treated for one year. The reference area showed no evidence of a decrease in density ($p = 0.6$) or frequency ($p = 0.66$), with a mean density change of 0.04 plants/m² (-0.29 to 0.36 , 95% CI) between 2016 and 2017 surveys. This pattern also occurred between the 2016 and 2018 surveys, with no evidence of a decrease in density ($p = 0.12$) or frequency ($p = 0.86$), and with a mean density change of -0.18 plants/m² (-0.49 to 0.13 , 95% CI; Supplemental material Table S2.1).

Typical of plant populations, density in each survey exhibited statistically significant ($p < 0.05$) positive spatial autocorrelation at sufficiently small radii. More importantly, however, the change in density after one year showed no statistically significant spatial autocorrelation, while

the change after two years showed no statistically significant spatial autocorrelation in the reference area and only weak positive autocorrelation in the Exp1 area (autocorrelation at radius ≤ 50 m). Therefore, the spatial independence assumption of our statistical tests for treatment effects using the point-intercept data is valid in all three areas for one year of treatment and in the reference area for two years of treatment, and it is an acceptable approximation for two years of treatment in the Exp1 area because the p value for our hypothesis test is well below the significance level.

Timing of Treatment. Efficacies of early and mid-season herbicide treatments were significantly different from one another ($p < 0.03$), with the mid-season efficacy being higher. Mid-season herbicide treatment was also significantly more effective than early-season manual treatment ($p < 0.006$). There was no significant difference in efficacy of manual removal based on timing (early, mid and late season), nor did the efficacy of mid-season and late-season herbicide treatments differ significantly (Figure 2.3). When comparing treatment timing and type (manual and herbicide) with the reference plots, we found significant differences for both mid-season treatments (herbicide $p < 0.001$, manual $p = 0.05$). When we examine each season for a specific treatment type, we see that treatment was not effective in altering *G. paniculata* density with either herbicide or manual treatment in the early-season (June 13 to June 23). The treatments do show a divergence in density reduction in mid-season, with a large increased effectiveness of herbicide during this time (Figure 2.3). The reference group had a small positive density change, showing that there were no strong environmental factors causing reductions in density, and reductions detected in the experimental plots can therefore be attributed to the treatments.

Resprout Following Treatment. Resprouting of marked plants within one growing season of treatment was seen in 10% (4-22%, 95% binomial CI) of the manually removed plants and 0% of the herbicide-treated plants (Figure 2.4). In 2018, one year following treatment a similar trend emerged, with 14% (7-26%, 95% binomial CI) manually removed plants and 2% (0-11%, 95% binomial CI) herbicide-treated plants resprouting. When digging down to the caudex and taproot of the manually removed plants exhibiting resprouting, we saw resprouting both when a portion of the caudex was left and when the entire caudex was removed. All marked plants in the reference area survived the season in 2017 and resprouted in 2018.

For the large-sample test for the difference between manual and herbicide removal methods one year after treatment, we found a significant difference in the chance of resprouting for manual removal when compared with herbicide ($p < 0.027$, 0.01-0.24, 95% CI) and resprouting had a higher chance of occurring in the marked reference plants that did not receive treatment when compared to both manual and herbicide removal methods ($p < 0.001$, reference-manual: 0.68-0.93, reference-herbicide: 0.82-1.0, 95% CI).

Discussion

Gypsophila paniculata is a problem invasive species in much of the northern US and southern Canada, but relatively little is known about its response to treatment on large spatial scales (multiple hectares), the time of year when treatment is most effective, or its potential to resprout following treatment. Our results show that one and two years of *G. paniculata* control reduced, but did not eliminate the plant's presence, that the use of herbicide is more effective than manual removal in reducing plant density and resprouting, and that treatment is most effective in reducing *G. paniculata* density in northwest Michigan when administered in late June and early

July.

Gypsophila paniculata density was reduced slightly after one and two years of treatment in both Exp1 and Exp2 areas, but a reduction in frequency of occurrence was detected only in the Exp2 area. In both instances, the results reinforce the notion proposed in previous studies that managers of *G. paniculata* must retreat areas for several consecutive years to achieve substantial reductions in abundance (Loope and Siterlet 2000, Emery et al. 2013). Based on visual inspection, it appears that regrowth in the Exp1 area after two years of treatment occurred mainly from immature plants that were missed by treatment crews and from new seedlings, with most large mature *G. paniculata* plants having been successfully eliminated. A study by Emery et al. (2013) that employed 0.1-hectare experimental plots found manual removal reduced *G. paniculata* cover by 30% following one year of treatment.

For the timing of treatment survey, we found that treatment was least effective at the beginning of the management period (mid-June). However, treatments for the resprout study were also administered in early June, and the results clearly show that treatments of individual plants at this time were highly effective. For that reason, we propose the lack of early-season treatment effectiveness in the surveys may be due to a relatively high proportion *G. paniculata* plants being missed by treatment crews. At this stage in their growth, immature *G. paniculata* plants up to two years of age easily blend in with other vegetation, particularly with confamilial *Silene vulgaris* (bladder campion), which looks nearly identical to *G. paniculata* at this time of year. Another related consideration is that seedlings (around 6 cm tall by mid-June) and immature plants (around 12 cm at one year) may be overlooked due to their small aboveground growth (Darwent and Coupland 1966). We propose that managers take additional time to educate removal crews on the appearance of small *G. paniculata* plants if treatment is performed in early

to mid-June, or preferably delay treatment until late June when *G. paniculata* can be identified more rapidly and reliably.

Herbicide treatments administered from late June to early July were particularly effective at reducing *G. paniculata* density. While further study is required to understand the mechanism that drives such a pronounced response to glyphosate at this phenological stage, a few possible explanations are apparent. Between late June and early July *G. paniculata* plants are producing large amounts of new biomass and shuttling stored carbohydrates from the taproot for flower production (Darwent 1975). In other plant species, herbicide has been found to be more effective when administered at this stage of development due to the increased abundance of carbohydrates in the above ground biomass (Derr 2008, Mozdzer et al. 2008). Herbicide was less effective from mid to late July, producing a smaller and more variable reduction in density than the mid-season herbicide treatment. This may be due to *G. paniculata* increasing sequestration of carbohydrates (with the applied herbicide) from the shoots to the taproot for overwinter storage (Marks et al. 1994, Hallinger and Shisler 2009).

Another consideration is that the late-season treatment is less effective following the production of seeds that may germinate in subsequent years. Rice et al. (unpublished research) found that the later the herbicide treatment is administered, the less effective it becomes at preventing the development of seeds to maturity in the parent plants. Additionally, manual removal should be done with care during the late-season because it may aid in the dispersal of seeds when plants are severed and left on site.

One benefit to treating *G. paniculata* from late June onward, however, is that the plant has reached its visually distinctive stage and the stems are beginning to change from green to purple, making it easier to identify. In early to mid-July, the plants are also starting to produce

thousands of gray buds which further differentiate them from neighboring plants. From this stage through the end of the treatment season, mature *G. paniculata* plants are easily identified for treatment by removal crew members.

We found resprouting occurred both in manually removed plants and, less frequently, in herbicide-treated plants. Prior to this study, it was unknown whether these treatments were effective at preventing resprouting. It was thought that *G. paniculata* was not able to regenerate stem tissue from the taproot (Loope and Siterlet 2000, Emery et al. 2013), and resprouting of manually removed plants was therefore attributed solely to incomplete removal of the caudex from the taproot during manual removal. However, horticulturalists commonly propagate *G. paniculata* and other *Gypsophila* perennials from root cuttings (PFAF, GPPD). One year after treatment, it appears that herbicide treatment is more effective at preventing resprout than manual removal and should be used preferentially when treating a large infestation. The one herbicide treated plant that did exhibit resprouting one year following treatment was the only herbicide marked plant not sprayed completely in 2016; when plants were checked at the end of the season, one stem of this plant was still healthy. For that reason, we attribute resprouting to insufficient herbicide application, and we suggest that thorough coverage of a plant's foliage is necessary to ensure that resprouting does not occur.

Conclusions and Management Implications. Our results show that one or two years of treatment for *G. paniculata* infestations reduced but did not eliminate plant presence, and that the use of herbicide provided more effective control than manual removal methods. While resprouting and aided seed dispersal are concerns with manual removal, it remains a good alternative to herbicide with the reduced potential of damage to the surrounding plant community

and lower cost. The optimal time to treat with manual removal in northwest Michigan is from late June to early July; if completing treatment in this short time frame is not feasible (e.g., with large infestations), managers should attempt to complete treatment before seeds mature (mid to late July) to minimize facilitation of seed dispersal. Herbicide application is more effective than manual removal in preventing resprouting, and the optimal treatment time is from mid-June to early July. Although this study was conducted in northwest Michigan the results likely apply more broadly to areas with similar climate and soil composition. *Gypsophila paniculata*, although a monoculture in many parts of SBDNL, is still restricted to a relatively small portion of Michigan's extensive coastal dune system. If the small satellite populations are treated quickly and effectively, the result should be a more manageable infestation and prevention of further spread, protecting the unique Great Lakes native dune plant community.

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Figures

Figure 2.1. Study site including Experimental Area 1 (Exp1), Experimental Area 2 (Exp2) and reference areas in Sleeping Bear Dunes National Lakeshore (Leelanau County, MI). All study sites had similar initial *G. paniculata* density of approximately 2 plants/m².

Figure 2.2. Box plot of point-intercept density change before treatment and after treatment in the three study locations after one and two years of treatment over the course of the three-year study. We found a statistically significant decrease in density of *G. paniculata* after one year of treatment in Exp1 and Exp2 ($p < 0.001$) while the reference area showed an increase in density ($p < 0.001$). The same trend emerged after two years of monitoring in the Exp1 and reference areas. Gray horizontal reference line at zero.

Figure 2.3. Change in *G. paniculata* density from 2016 to 2017 in the Exp1 based on the timing of treatment by group and the type of treatment (herbicide and manual). Early: June 13 – June 23, Mid: June 27 – July 7 and Late: July 11 – July 27. The dashed horizontal line represents the reference plots' mean density change; the dotted horizontal lines indicate the 95% confidence interval. Groups labeled with different letters exhibit statistically significant pairwise differences.

Figure 2.4. Probability of *G. paniculata* resprout based on treatment type. Treatment was applied in early June of 2017 to 100 plants in Exp2 and 25 plants were marked in the reference area. Bars represent 95% binomial confidence intervals.

Chapter 3

Phenology of Seed Maturation in *Gypsophila paniculata* and Its Relation to Glyphosate Efficacy

Abstract

Baby's breath is an aggressive invasive plant in large parts of Canada and the northern United States. It reproduces and disperses mainly by seed, but the phenology of seed maturation is poorly understood. We conducted a study to document the phenology of seed maturation and assess its relevance to an ongoing restoration program in a coastal sand dune system in northwest Michigan. Seeds were collected on five dates during July and August in both 2016 and 2017 to test for germinability. Percent germination increased from 20% to 81% between collection dates of July 22 and 28 and exceeded 90% by August 4, 2016. A similar trend occurred in 2017. To assess dependence of glyphosate efficacy in preventing seed maturation on the timing of application, we tested germinability of seeds from plants sprayed with glyphosate on three dates in July 2016. Percent germination increased from 0% to 20% for treatment dates between July 11 and 25, highlighting the importance of completing treatment early in the growing season.

Key words: Invasive, management, percent germination, sand dune, weed.

Restoration Recap

- *Gypsophila paniculata* is a problem invasive in many parts of North America, but relatively little is known about the phenology of seed maturation in its introduced range.

This study investigates the phenology of maturation and its relation to glyphosate efficiency in the sand dunes of northwest Michigan, the site of a large infestation.

- Seed maturation of *G. paniclata* exhibits a consistent phenology, with rapid increase in germinability near the end of July and attainment of maximum germinability by the end of August.
- Early glyphosate application (before mid-July) prevents the production of viable seeds. Late glyphosate application is less effective, though it still reduces germinability.

Introduction

Invasive species are defined as non-indigenous species that threaten biological diversity and cause or are likely to cause economic or environmental injury in their introduced range (Beck et al. 2006, CBD 2008). Negative consequences from invasive species cost countries around the world billions of dollars annually in reduced agricultural production, degradation of natural spaces and concomitant losses of income from tourism and recreation, and reduction of overall biodiversity (Pimentel et al. 2005, Pejchar et al. 2009, Powell et al. 2011, Vilà et al. 2011). For this reason, management of invasive species is often a restoration priority. However, in many cases the best management strategy for control of an invasive species is unknown due to a lack of information on the basic biology of the problem plant.

Gypsophila paniculata (baby's breath) is an herbaceous perennial plant in the family Caryophyllaceae that is native to Eurasia. It was first introduced to North America in the 1800s and has become invasive in large parts of Canada and the northern United States (Darwent and Coupland 1966) where it can be found growing in meadows, sand dunes, farm fields and roadsides (Darwent 1975). *Gypsophila paniculata* out-competes native plants in sandy, well-

drained soils due in part to its deep taproot (up to four meters), giving it access to scarce resources (Darwent 1975, Karamanski 2000). In coastal dune habitats of northwest Michigan, *G. paniculata* can be found in dense stands of 50% to 80% ground cover with an average density of two plants/m² and has been shown to reduce native species abundance and increase the abundance of non-native species (Karamanski 2000, Emery et al. 2013, E. Rice, Grand Valley State University, unpub. data). The area that *G. paniculata* infests in Michigan is a part of the largest freshwater dune system in the world and home to several threatened, endangered and endemic species (Albert 2000). For these reasons, *G. paniculata* is a priority invasive species for detection and control in coastal habitats of northwest Michigan (DNR 2015).

Gypsophila paniculata infestations in northwest Michigan typically are managed with a combination of manual removal and foliar application of glyphosate, depending on wind conditions and whether state or federally protected plant species are present (TNC 2013). Manual removal consists of using a spade to sever the taproot below the caudex (where stems meet the root), which is thought to prevent resprouting (Loope and Siterlet 2000). Glyphosate, while a more efficient method of removal in densely infested areas, must be applied with care due to its non-selective nature. Between 2003 and 2013 removal crews dedicated over 45,000-person hours and treated over 670 ha in coastal dune habitats in northwest Michigan (TNC 2013). Treatments have been shown to reduce *G. paniculata* cover from 50% to less than 10% in some treated areas (Emery et al. 2013), but regrowth occurs in treated areas and eradication is no longer considered a realistic goal. Given the constraints of both methods, the current management strategy is therefore to control existing populations and prevent further spread.

Field crews typically begin work in early June when the plants are approximately 25 cm in height and plants may be reliably distinguished from *Silene vulgaris* (bladder campion), a

similar confamilial species. Flowering of *G. paniculata* begins in early July and fruits begin appearing from mid to late July, well before field crews have completed their management activities in mid-August. Seeds germinate in the spring from May to June under natural conditions and do not exhibit dormancy when tested (personal observation, Darwent and Couplant 1966).

Determining the phenology of seed maturation is an integral part of managing invasive weeds that reproduce sexually. *Gypsophila paniculata* produces a large number of seeds annually (ca. 14,000 per plant: Darwent and Couplant 1966), which are dispersed in a tumbleweed fashion over distances of up to one kilometer as wind breaks the base of brittle plant stems from late August onward (Darwent 1975). It is therefore desirable to mechanically remove plants or spray them with glyphosate before seeds mature to reduce the rate at which infestations spread into new areas as well as the rate at which treated areas are recolonized. While at least one study has attempted to determine optimal environmental parameters (temperature, light, burial depth, and soil texture) for germination of mature seeds under controlled laboratory and greenhouse conditions (Darwent and Couplant 1966), no studies have yet been conducted to determine the phenology of seed maturation in invasive populations of *G. paniculata* in the field.

A related management issue is the efficacy of glyphosate in preventing maturation of *G. paniculata* seeds. This is important to determine because glyphosate is preferentially used for *G. paniculata* control in Michigan whenever field conditions permit. Glyphosate is a nonselective systemic herbicide that is readily translocated to developing fruits, where it can reduce seed germinability and vigor (Baig et al. 2003, Piotrowicz-cieślak et al. 2010). Its effectiveness, however, varies with plant species and timing of application. For example, glyphosate has minimal or no effect on the germinability of *Poa pratensis* (Kentucky bluegrass), *Festuca rubra*

(red fescue), and *Sida spinosa* (prickly sida) (Klingman and Murray 1976, Egley and Williams 1978), whereas *Xanthium strumarium* (common cocklebur), *Sesbania exaltata* (hemp sesbania), and *Glycine max* (soybean) show dramatic reductions (Clay and Griffin 2000, Jeffery et al. 1981). To date, no studies have been conducted to determine the effects of glyphosate treatment on seed development and germinability in *G. paniculata* or the dependence of these effects on the timing of application.

This study was conducted to answer two questions about *G. paniculata* seeding: what is the phenology of seed maturation in a coastal dune habitat in northwest Michigan? And how is germinability affected by the timing of glyphosate application?

Methods

Study Sites and Seed Collection. All seeds were taken from a 40-ha area in the plateau region of Sleeping Bear Dunes National Lakeshore in northwest Michigan, USA (44.872°N, 86.057°W). This area has a *G. paniculata* cover ranging from 25-50% (E. Rice unpub. data) with no history of prior management. Average annual mean temperature (2016: 10°C, 2017: 9°C, historical: 9°C) and average annual precipitation (2016: 32 in, 2017: 33 in, historical: 32 in) were consistent across years falling within 1998 to 2017 historical ranges (Supplemental material Table S3.1; MRCC 2018).

For the 2016 collection, seeds were harvested on five dates between July 22 and August 23 from 16 untreated plants within a 20-ha area to determine the phenology of seed maturation. Seeds were also collected on August 23 from 38 plants that had been treated via foliar application of 23.4 ml L⁻¹ glyphosate (Roundup ProMax®, Monsanto) on three different dates between July 11 and July 25, 2016 (one treatment per plant). In 2017, to avoid collecting from

previously treated plants, seeds were collected north of the 2016 collection site with similar topography and plant community. Seeds were collected on five dates between July 26 and August 22 from 20 untreated plants.

Seeds on plants from which collections were taken were abundant but not quantified. Seeds collected from each plant on each collection date were placed in separate paper coin envelopes and stored in the dark from 21°C to 23°C in sealed bags containing silica gel beads until the germination experiments were run.

Germination Experiments. To ensure that all seeds tested could be checked for germination (radicle emergence) daily within a three-hour window, a subset of seed samples from each year was randomly selected for the experiment. Specifically, seeds from five of the 16 untreated parental plants from 2016 were used for the phenology of maturation experiment, while seeds from 12 of the 38 treated parental plants were used for the maturation with glyphosate experiment. Seeds from 12 of the 20 untreated parent plants from the 2017 collection were used. In all germination experiments, 100 seeds collected from each parental plant on each collection date were placed on moist filter paper (MN 410) in 94-mm x 16-mm Petri dishes and incubated in a growth chamber at 20°C and 112 $\mu\text{mol m}^{-2}\text{s}^{-1}$ photosynthetically active radiation from florescent bulbs with a 12:12 h light:dark photoperiod. A total of 2,500 seeds were tested from the 2016 collection (100 seeds/plant x 5 plants/collection date x 5 collection dates) and a total of 6,000 seeds from the 2017 collection (100 seeds/plant x 12 plants/collection date x 5 collection dates) to determine the phenology of maturation.

Each day for 12 days, the same individual checked the Petri dishes (to minimize bias) and newly germinated seeds were counted and removed. Germination was operationally defined as

radicle emergence (Baskin & Baskin 2001). Petri dish positions in the growth chamber were randomized daily to avoid potential biases in temperature or light exposure. To show that experimental conditions were adequate, commercial seeds known to have germination percentages in excess of 90% were also tested. The commercial seeds used were of the Early Snowball cultivar distributed by W. Atlee Burpee & Co and were produced in the Netherlands in 2016. The same procedure was employed in the maturation with glyphosate experiment except that each parent plant was represented by one set of 100 seeds with different treatment dates and collected on the same date on August 23, 2016; a total of 1,200 seeds were tested (100 seeds/plant x 4 plants/treatment date x 3 treatment dates).

Statistical Analysis. For each collection date of untreated plants (phenology of seed maturation) and glyphosate treatment date of treated plants (maturation with glyphosate), the temporal pattern of germination during the 12-day experiment was characterized by fitting a nonparametric Kaplan-Meier time-to-event curve (McNair et al. 2012). Statistical Kaplan-Meier time-to-event curves are an estimate of the probability that the event in question (in this case, germination) has *not yet* occurred, as a function of incubation time. In our experience, however, most biologists find it more intuitive to think in terms of the probability that the event *has already* occurred, which is one minus the probability that it has not yet occurred. We call this relationship the complementary Kaplan-Meier curve, and it is the relationship we show in all plots of the results. Germination patterns for different collection dates were compared pairwise using log-rank tests with Holm correction for multiple comparisons, as were germination patterns for different glyphosate treatment dates (McNair et al. 2012). The Kaplan-Meier log-rank test assumes all seeds share the same probability distribution for time to germination, and

that this test also assumes seed germination times are mutually independent. It was not feasible to test these assumptions, but we believe our analysis is appropriate for purposes of this exploratory study. All statistical analyses were performed using R software, version 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Phenology of Maturation. Seeds from untreated plants show a clear trend of increasing germinability with successive collection dates (Figure 3.1). There were statistically significant differences (log-rank test; $p < 0.001$) in germination patterns for all pairs of collection dates except the final two in August for both 2016 and 2017 ($p = 0.71$, $p = 0.093$) (Supplemental material Table S3.2 and Table S3.3). In 2016 percent germination increased markedly from 20% (16–23%, 95% confidence interval) to 81% (77–84%, 95% confidence interval (CI)) in seeds collected between July 22 and July 28 and increased to 97% (95–98%, 95% CI) the August 23 collection (Figure 3.2). A similar trend emerged in 2017, with percent germination starting at 15% (13–17%, 95% CI), increasing to 74% (71–76%, 95% CI) the following week, increasing further to 95% (94–96%, 95% CI) by August 16, and declining slightly on August 22 to 90% (88–91%, 95% CI) (Figure 3.1; Figure 3.2), though the statistical significance of this decline is not supported by the log-rank tests. In both years, then, the germination rate increased for successive collection dates until plateauing at or above 90%.

Maturation with Glyphosate. Seeds from plants sprayed with glyphosate on three different dates in July 2016 showed a pattern of increasing germinability with later treatment date (Figure 3.3). The results showed statistically significant pairwise differences between all three pairs of

treatment dates (Supplemental material Table S3.4), with 0% germination in seeds from plants treated in early-July, 13% (10–16%, 95% CI) in plants treated in mid-July, and 20% (16–23%, 95% CI) in plants treated in late July.

Discussion

Gypsophila paniculata is an aggressive invader of coastal dune habitats in northwest Michigan and in many other locations in the northern United States and Canada. In well-established populations, this plant is difficult to eradicate. Therefore, managers should consider the plant's basic biology to determine how to optimize management strategies. This study provides the first assessment of seed maturation phenology for an invasive population of *G. paniculata*.

We found that phenology of seed maturation was reasonably consistent among seeds collected in 2016 and 2017. There were significant differences between the first three collection dates as germination percent continually rose, until the final two collections when the germination percent was not significantly different. Seed maturation exhibited a rapid increase in germinability near the end of July and attainment of maximum germinability by the end of August. We did, however, see that onset of the rapid increase in seed germinability was delayed somewhat in 2017 compared to 2016 (Figure 3.2), which we suspect was due mainly to lower temperatures in 2017 and consequently a lower rate of accumulation of growing degree days. Like many physiological processes, the rate of plant growth and development is strongly positively related to temperature over the range of temperatures normally experienced in the field. However, plant growth and development essentially cease when ambient temperature falls below a threshold, commonly taken to be 10 °C. Growing degree-days (GDDs) are a combined measure of how long and by how much the ambient temperature lies above this threshold. In

temperate climates, cumulative GDDs between January 1 and any particular subsequent date are a useful indicator of how much plant growth and development is likely to have occurred. Based on cumulative GDD data from the Midwest Regional Climate Center for a nearby monitoring station (MRCC 2018), it appears that GDDs accumulated more slowly (the temperature was cooler) in 2017 than in 2016 in relation to their field collection date (Figure 3.4). This suggests that phenological milestones would have been reached later in 2017, consistent with the pattern observed for seed maturation. The earlier a higher accumulation of GDDs is acquired, the sooner seeds will be able to germinate. The rapid increase in seed germinability in 2017 occurred about five days later than in 2016 (Figure 3.2), mirroring the roughly 5-day shift in the cumulative GDD curve (Figure 3.4) for the corresponding time of year in 2017 compared to 2016. Thus, the slight shift we see in the timing of seed maturation in 2017 can be explained by the slower accumulation of GDDs.

In addition to understanding the phenology of seed maturation, we are also interested in how glyphosate influences germination of *G. paniculata* because unlike many other invasive plants, such as Japanese knotweed (Hollingsworth and Bailey 2000) and Eurasian watermilfoil (Smith and Barko 1990), it is thought that *G. paniculata* disperses primarily via seed (Darwent and Couplant 1966). At our study site in 2016, herbicide treatment was more effective in preventing production of viable seeds when glyphosate application occurred earlier in the growing season and seed maturation process. This outcome is consistent with other studies that found that when glyphosate is applied during early phases of seed maturation, the herbicide accumulates in the seed and results in incomplete development of the endosperm and embryo and reduce seed viability (Cessna et al. 2002, Baig et al. 2003). Glyphosate application in our study area ended on July 25, by which time seed development had proceeded far enough so that

roughly 20% of the seeds were germinable. As seed maturation progresses, the effectiveness of glyphosate appears to decline, a trend seen in other plant species receiving late glyphosate application (Clay and Griffin 2000, Bennett and Shaw 2000). Although 20% of seeds from plants treated on July 25 germinated, this is still an improvement compared to the 97% germination of seeds collected from untreated plants on the same date (August 23, 2016).

Seed maturation of *G. paniculata* in northwest Michigan had a consistent phenology, with rapid increase in germinability near the end of July and attainment of maximum germinability by the end of August. To optimize control efforts with both manual and herbicide application, treatment of *G. paniculata* should conclude by mid-July. Manual removal after this time is likely to aid seed dispersal. If treatment does continue through July, glyphosate should be used exclusively, because early July glyphosate application reduces the production of viable seeds. Managers must be cognizant, however, that the later glyphosate is applied, the less effective it becomes at reducing germinability. To improve control efforts, managers should be aware of *G. paniculata*'s maturation phenology and its relation to glyphosate efficiency to prevent this increasingly problematic invasive species from spreading throughout Michigan and other areas in North America.

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Figures

Figure 3.1. Complementary Kaplan-Meier time-to-germination curves for *G. paniculata* seeds collected from untreated plants at SBDNL to determine the phenology of maturation. (A) Left: Five collection dates in 2016. All germination patterns for different collection dates were statistically significantly different ($p < 0.001$) with the exception of those for August 12 and 23 ($p = 0.71$). (B) Right: Collection dates in 2017. Time-to-germination curves exhibit the same trend as the previous year; all germination patterns for different collection dates were statistically significantly different ($p < 0.001$) with the exception of those for August 16 and 22 ($p = 0.093$).

Figure 3.2. Final percent germination (day 12) from figure 1 of untreated *G. paniculata* plants as a function of day of the year on which the seeds were collected. The bars represent pointwise 95% confidence intervals. Day 205 is July 23, 2016 and July 24, 2017.

Figure 3.3. Complementary Kaplan-Meier time-to-germination curves for *G. paniculata* seeds collected on the same day (August 23) from plants that had been sprayed with glyphosate on three different dates in 2016. All germination patterns for different treatment dates were statistically significantly different from one another (largest $p = 0.0077$).

Figure 3.4. Cumulative growing degree-days versus day of year in 2016 and 2017. The gray band represents the range of cumulative growing degree-days for each day of the year over the 20-year period from 1998 to 2017. The horizontal line serves as a visual aid to highlight the shift or delay (ca. 5 days) in cumulative GDDs for 2017 compared to 2016 around day 210 when seeds become fully mature. Cumulative growing degree-days data are from the Frankfort 2NE monitoring station located 27 km south of the study site, with a temperature threshold of 10°C (MRCC 2018).

Chapter 4

Extended Literature Review

Invasion

Successful invader characteristics. Few invaders successfully establish in an introduced range, but those that do and outcompete native plants can have devastating effects. The likelihood of invasion success is highly dependent on the number of habitats the plant species occupies in its native range and the residence time in the invaded range (Kleunen et al. 2015; Pysek et al. 2015). If the species is more of a generalist in its native range, it will be well suited to more potential introduction sites. The longer a species is present in its introduced habitat, the more likely it is to become invasive. Some of the main biological characteristics associated with plants becoming invasive include a persistent seed bank, long flowering periods, stress tolerance, high seed output, multiple dispersal vectors, vegetative reproduction, self-fertilization, and rapid growth (Kleunen et al. 2015; Pysek et al. 2015). These characteristics enable the plant to be competitive and proliferate effectively.

The lack of specialized soil pathogens and herbivores initially allow invasive species to thrive in an introduced environment unencumbered by disease and predation (Dostal et al. 2013). Over time, pathogens from native pests start to predate on invasive species, reducing their competitive advantage over native species. An example of this boom-and-bust cycle was seen in *Heracleum mantegazzianum*. Analyzing grasslands that had been invaded at different times, Dostal et al. (2013) determined that native species richness and productivity recovered after 30 years of *H. manegazzianum*'s residence in the invaded area. This study showed that the initially superior competitive ability of an invasive species can later be overturned by stabilizing

processes such as negative plant-soil feedback (Dostal et al. 2013). How commonly this phenomenon occurs, however, is unknown.

Herbicide resistance. One of the most effective ways to control problem invasive species is through herbicide application. This method is particularly effective on weedy species and is among the most efficient for managers. Unfortunately, because of the popularity of herbicides and their subsequent heavy use, herbicide resistance has evolved in many plant taxa. Although resistance has not yet been reported in *Gypsophila paniculata*, the invasive species of interest in this study, it is a serious concern due to the same herbicide (glyphosate) being used for consecutive years over the last decade. There are now over 200 species that have evolved herbicide resistance due to heavy reliance on a limited number of herbicides created over the last 65 years (Heap 2014). Herbicide resistance is most often a result of natural selection due to rare mutations existing in a population prior to treatment, which enable the plant to be partially tolerant to the chemical being applied. With repeated herbicide applications, these rare mutations become more frequent in the population, due to higher fitness when compared to susceptible plants (Heap 2014).

Five key mechanisms for herbicide resistance have been identified: target-site resistance due to enzymes preventing binding of the herbicide to the site of action, heightened metabolism allowing the herbicide to be processed before the plant dies, decreased translocation of the herbicide to the target site, sequestering of the herbicide in vacuoles or cell walls, and duplication of the gene coding for the target enzyme, causing increased production of the enzyme and hence reduced effectiveness of the herbicide (Heap 2014, Sammons and Gaines 2014). The evolution of herbicide resistance in plants is influenced by gene mutation, gene duplication, initial frequency of resistant alleles, effective population size, the degree of

dominance of resistant alleles, epistatic interactions, the mode of reproduction, and weed fitness when herbicide is present as well as when it is absent (Jasieniuk et al. 1996; Renton et al. 2011). Once a resistant mutant surfaces in a population, the repeated use of a herbicide with the same mode of action will favor rapid evolution of resistance within the population. The number of generations it takes to reach a majority resistant population is determined mainly by the initial frequency of resistant alleles and the magnitude of the selective advantage they confer. Resistance to all herbicides (except triazine via cytoplasm inheritance) is transmitted through nuclear inheritance via the ovules and pollen (Jasieniuk et al. 1996). There can also be a fitness cost associated with herbicide resistance when compared to susceptible plants in the absence of herbicide application (Jasieniuk et al. 1996). One study looked at glyphosate resistance in *Ipomoea purpurea* and found that highly resistant populations had shorter roots, lower germination rates, and smaller aboveground biomass (Etten et al. 2016). These tradeoffs make it less competitive in terms of nutrient acquisition and reproductive output when the herbicide is not present. If the same herbicide is applied frequently, the fitness gain when the herbicide is present outweighs the fitness loss when it is absent.

Resistance to Glyphosate. Glyphosate is a widely used herbicide in both agriculture and land management and is the only herbicide currently used in northwest Michigan to treat *G. paniculata*. It is a valuable herbicide due to its reasonable price and the low risk of herbicide resistance it is thought to pose. Glyphosate is considered to be a low-risk herbicide because it acts upon fewer target site mutations that have been discovered to cause resistance when compared to other herbicides (Heap 2014). That being said, there is still a chance of herbicide resistance becoming problematic, because glyphosate is used widely and on many weed species. Due to our recent heavy reliance on glyphosate, to date there are 24 weed species that have been

documented as glyphosate resistant since it became commercially available in 1974 (Heap 2014). Two of the most concerning and economically detrimental of these weeds are *Amaranthus palmeri* and *Amaranthus tubercularthus*, because they infest roundup-ready crops and previously evolved resistance to other herbicide sites of action (Heap 2014). These species, among others, are now considered super weeds. Unfortunately, there have been no new herbicide discoveries pursuing a novel target site in 30 years to combat this growing problem (Heap 2014).

A related management issue is the efficacy of glyphosate in preventing maturation of seeds and whether seed maturation is influenced by the timing of glyphosate application. Glyphosate is a nonselective systemic herbicide that is readily translocated to developing fruits, where it can reduce seed germinability and vigor (Baig et al. 2003, Piotrowicz-cieślak et al. 2010). Its effectiveness, however, varies with species and timing of application. For example, glyphosate has minimal or no effect on the germinability of *Poa pratensis*, *Festuca rubra*, and *Sida spinose* (Klingman and Murray 1976; Egley and Williams 1978), whereas *Xanthium strumarium*, *Sesbania exaltata* and *Glycine max* show dramatic reductions (Clay et al. 2000, Jeffery et al. 1981). The variety in the effects of glyphosate on the seeds of different species is very interesting, but more investigation needs to be done into the mechanisms that trigger these disparate responses.

Northwest Michigan dune system

Sleeping Bear Dunes National Lakeshore (SBDNL). SBDNL is part of the largest freshwater dune system in the world and is an important economic and ecological resource for northwest Michigan (Albert 2000). Recreation and tourism in this region result in around 1.5

million visitors annually, which generates over \$800 million dollars in revenue and supports over 2,000 jobs (Cui et al. 2011).

Abiotic factors. The dunes in the Great Lakes region were created by receding glaciers that deposited large quantities of sand, silt, and gravel from their flanks that accumulated into dune structures (Albert 2006). The main concentration of sand collects at the mouths of rivers and on coastal bluffs. These dune complexes, found all along the eastern side of Lake Michigan, are both shaped and eroded by the wind, prevailing current, and lake levels (Albert 2006). Aeolian sand (transported by wind) is the only type of sand found at our study site on the dunes plateau in SBDNL (NRCS). The hostile dune environment is defined by extreme summer (hot) and winter (cold) temperatures, high wind speeds, minimal nutrient availability, and alkaline well-drained sandy soil (Albert 2006).

The dune system is naturally disturbed due to the unstable nature of sand formations, making it more vulnerable to invasive species. This instability is exacerbated by human development and disturbance. Invasive species are thought to be less likely to succeed in dune habitats if they have a diverse and stable plant community (Rudzite 2002). But once established in the dunes, invasive species chemically and physically alter soils, and these changes persist following the plants' removal (Sawai and Saito 2011). This is especially true following manual removal, where the soil has been disturbed and exposed to more light, and temperature fluctuation increases along with nitrogen mineralization rates (D'Antonio and Meyerson 2002). This sets the stage for fast growing opportunistic secondary invaders to gain a foothold.

Biotic factors. The dune system at SBDNL is home to many endangered and endemic species, including *Charadrius melodus* (Piping plover), *Trimerotropis huroniana* (Lake Huron locust), and *Cirsium pitcheri* (Pitcher's thistle). The foredune and secondary dune, just past the

beach and before the tree line, contain the highest concentration of endangered and endemic species in the dune system (Albert 2006). These areas are also where many invasive species are found, including *Silene vulgaris* (Bladder campion), *Centaurea maculosa* (Spotted knapweed), and *G. paniculata* (Baby's breath). *G. paniculata*, a recently listed priority invasive species by the Michigan Department of Natural Resources (DNR 2015), is most prevalent within SBDNL, posing a formidable threat to many unique species found in this habitat (TNC 2013). *Gypsophila paniculata* possesses many survival advantages that have enabled it to infest about 25% of SBDNL; in some of those areas *G. paniculata* comprises over 80% of the vegetation (Vandermeulen 2006).

In dune habitats, seed banks are typically short lived but need to be taken into consideration prior to restoration efforts (D'Antonio and Meyerson 2002). The aboveground vegetation does not always mirror what is present in the seed bank (D'Antonio and Meyerson 2002; Leicht-Young et al. 2009). In regards to native plant restoration, unless a source population is in close proximity, it is unlikely that the species will be present elsewhere in the dune system seed bank. As a result, restoration efforts can be stunted, especially following an invasive plant becoming established and depleting the native species seed bank (French et al. 2011; Leicht-Young et al. 2009).

***Gypsophila paniculata* biological information**

Invasion history. *Gypsophila paniculata* is an herbaceous perennial in the family Caryophyllaceae, native to the Black Sea region of Eurasia. It was first introduced to North America in the late 1800s as a garden ornamental in Manitoba, Canada (Darwent and Coupland 1966) and was initially documented in northwest Michigan in Emmet County in 1913 (Gleason

s.n. MICH). Around SDBNL, *G. paniculata* was first observed at Point Betsie in the 1950s (Kleitch 2009). Since that time, it is thought that *G. paniculata* has spread predominantly to the northeast along 260 kilometers of Lake Michigan's shoreline. Certain life history characteristics of plants have been identified and associated with prolific invasion. Some of the indicators for high-risk invaders, especially agricultural invaders, include prolific reproduction, abiotic dispersal and lack of dormancy (Sakai et al. 2001). *Gypsophila paniculata* expresses all of these life history characteristics. Also aiding in its success in the northwest sand dunes of Michigan is its preference for dry, sandy, alkaline, and calcium-rich soil, allowing it to become one of the most aggressive and destructive invasive non-native plants threatening the region today (TNC 2013).

Seeds and seedlings. *Gypsophila paniculata* reproduces mainly by seed rather than vegetatively. It produces black seeds up to 2-mm in length that resemble snail shells. Seeds predominantly germinate in spring from May to early June and do not exhibit dormancy. According to Stevens (1957), an average-size plant produces approximately 14,000 seeds per year (we know of no estimates of lifetime reproduction for this iteroparous or polycarpic species). In comparison, the endangered and endemic *Cirsium pitcheri*, which is semelparous (monocarpic), produces between 50-300 seeds per plant (Hamze and Jolls 2000).

Germination is initiated when water is taken up by the seed and is completed with the rupturing of the seed coat and elongation of the radical (Bewley and Black 1985). Because the start of germination cannot be measured, most experiments define germination as having occurred when the radicle emerges (Baskin and Baskin 2001; Bewley and Black 1985). Based on lab experiments performed by Darwent and Coupland (1966), seeds germinate and grow in both fine and coarse textured sand, but have higher germination rates in coarse sand. They also found

that maximum germination takes place under a wide range of temperatures, from 10°C to 28°C, with the highest germination rate occurring at 20°C. *Gypsophila paniculata*'s ability to thrive in such a wide range of temperatures is yet another reason it is able to outcompete endemic species that are specialized to specific environmental conditions. It was also found that temperature affects the timing of germination; when seeds were exposed to 20°C conditions with continuous moisture, maximum germination was reached in two to three days, whereas at 10°C maximum germination did not occur until day five or six. Light presence or absence had no effect on total germination, but germination occurred more slowly under light conditions (Darwent and Coupland 1966).

When the radical emerges, it develops and extends rapidly from the lower end of the hypocotyl, followed within hours by the growth of root hairs. Two to three days after the completion of germination, the cotyledons appear and shed the seed coat. The seedlings grow quickly, producing three pairs of leaves in 16 days under ideal conditions. In field conditions the process is slower; secondary leaves appear after two weeks (Darwent and Coupland 1966). *Gypsophila paniculata* seedlings have a high mortality rate of 97% in the harsh dune habitat (Darwent 1975) By two years of age the immature plants are around 12 cm tall and the root is over 60 cm long (Darwent and Coupland 1966).

Taproot. *G. paniculata* can reach 0.75 m in height, but more impressively, its taproot can reach depths of up to 4 m (Darwent 1975). There are usually a few thin, short lateral roots just below the caudex, and the main root can branch at depths exceeding 60 cm. The long taproot enables *G. paniculata* to outcompete native plants readily and allows easy access to the scarce dune resources (Darwent 1975). Karamanski (2000) speculates that the taproot plays a role in reducing native plant species by over-stabilizing the dunes and preventing the natural shifting of

the sand that is necessary for seed burial and control of successional species. Although this is a popularly referenced view, there is little evidence to support it. One study measured changes in sand level following removal and did not find any change in sand stability when *G. paniculata* was absent (Emery et al. 2013). Moreover, the endemic dune species *Cirsium pitcheri* also produces a large tap root of up to 1.8 m in length (Weatherbee 2006).

The taproot is also where *G. paniculata* produces its secondary metabolites, the most prominent of which are saponins (Yao et al. 2010). Saponins make up an astounding 4% of the dry weight in *G. paniculata* (Korkmaz and Özçelik 2011). They are produced in the root and then transported throughout the plant. The saponins have antimicrobial properties, protecting *G. paniculata* from pathogenic microbes and herbivores (Sawai and Saito 2011). Unsurprisingly, some of the saponins are also found in the soil surrounding the plant and have an allelopathic impact on the neighboring plant and microbial community (Fons et al. 2003). People have found many uses for the saponins in *Gypsophila* species, especially in their native range. They are used in soaps, as a natural pest repellent, in fire extinguishers, to whiten gold, make cheese, and for some traditional medicinal applications (Pensec et al. 2013; Korkmaz and Özçelik 2011). This is one of the reasons that many *Gypsophila* species are endangered or threatened in Turkey where it is native (Korkmaz and Özçelik 2011).

General characteristics. Another *G. paniculata* characteristic of note is the woody caudex, or base of the plant where stem growth originates. This structure can reach 25 cm in diameter and the many stems protruding from it give the plant a globular bushy appearance (Darwent and Coupland 1966). If a stem breaks off before flowers are produced, the plant stem will resprout. The taproot lies about 4 to 7 cm below the caudex. Shoots begin to appear in late April and after two months the stems begin to become hollow (Darwent and Coupland 1966).

Gypsophila paniculata is synoecious perennial plant. Flowers are not produced for the first two years in natural settings, and flowering does not occur every year (Darwent and Coupland 1966). *Gypsophila paniculata* seems to be pollinated mainly by insects and to be both cross- and self-pollinating (Darwent and Coupland 1966). Fruit matures about four to five weeks after the first flowers bud, with a maximum of four seeds in each fruit. Typically, the ultimate unit, or the highest center flowers, produces four to three seeds, while the outer flowers usually produce one seed (Darwent 1975). As the fruit matures the stems become brittle, preparing to break off and tumble. One of the survival advantages of *G. paniculata* is its classification as a tumbleweed; its stems break off and roll, spreading seeds up to one km from the parent plant. Its dispersal is primarily wind-driven (Darwent 1975). Fruit usually opens to release seeds at the end of July, but this is dependent on each year's weather conditions (Darwent and Coupland 1966).

History of *Gypsophila paniculata* management

Gypsophila paniculata is a priority invasive species that has been undergoing removal in northwest Michigan since 1989, and in SBDNL since 2006 (Vandermeulen 2006). Between TNC, NPS, and the Grand Traverse Regional Land Conservancy, over 1,400 acres have been treated in 39,000-person hours (TNC 2013). As a result, *G. paniculata* cover has been reduced from 50% to less than 5% of vegetation cover in some treated areas (Emery et al. 2013). While considered a successful effort, about 1,900 infested acres of invasive baby's breath remain. Additionally, regrowth in treated areas continues to be a problem and requires continual maintenance. Crews, on average, treat just under eight acres a day (TNC 2013). Because *G. paniculata* is well established in the area, regrowth commonly occurs in treated areas, and resources (financial and human) are limited, the goal of eradicating *G. paniculata* is no longer

considered realistic and the emphasis has shifted to controlling existing populations and preventing further spread.

Reducing the abundance of *G. paniculata* is a priority for this area in part due to its presence being connected with reduction in the endangered and endemic pitcher's thistle and an increase in other non-native species (TNC 2013). This is cited by TNC as the main reason for removal, but the limited evidence gathered so far suggests that invaded areas do not show a lower total plant species richness or community diversity (Emery et al. 2013). Following treatment, however, Emery et al. (2013) found that native species that were present before treatment increased in abundance. The lack of change in total species richness can be attributed to the short lived or even absent seed bank of dune habitats and the need for longer term monitoring (Emery et al. 2013). French et al. (2009) argue that unless managers distribute seeds of desirable native species not already present, it is unlikely that species richness will increase following treatment.

The original goal set forth by TNC and NPS in 2006 was to contain *G. paniculata* spread and reduce the threat of invasion to a maintenance level by 2016 (Vandermeulen 2006). This goal was not achieved by 2016, but has been retained for the future. One additional objective is to reach out to land owners surrounding SBDNL to educate them about the threat *G. paniculata* poses. If permitted, crews will remove satellite populations from these private properties, reducing the risk of further spread and re-infestation (TNC 2013).

Before initiating the invasive species removal, managers need to take the time to understand how the species has altered the environment and how its subsequent removal could change the community structure (Emery and Doran 2013; D'Antonio and Meyerson 2002). It is a common practice to identify a non-native species as a problem without first considering how it

has adversely altered the environment or what financial commitment would be involved in the removal process. On the other hand, the benefits of the invader should also be considered. A few positive outcomes of *G. paniculata*'s invasion of the dune system have been discovered. In a study by Emery and Doran (2013), plots in which *G. paniculata* was present contained double the number of arthropods and 20% more arthropod families compared to plots that had low *G. paniculata* densities or from which it had been removed. Additionally, *G. paniculata* presence was shown to be coupled with an increase in sap-feeding herbivores, pollinators, and predator abundances. One caveat is that increased pollinator visits to *G. paniculata* were associated with reduced visitations to neighboring native plants (Emery and Doran 2013).

Manual removal. Over the years, various tests have been run to determine the best method of treating *G. paniculata*. Most of these trials were done by TNC or NPS and have not been published or reported. TNC's tests of alternative treatment methods have included cutting, burning with a hand torch, and application of glyphosate. Based on these tests, TNC concluded that manual removal by cutting *G. paniculata* is the most effective treatment method, provided the root is severed well below the caudex; they think if the plant is not cut below the caudex, rapid resprouting occurs within treatment plots (Loope and Siterlet 2000; Vandermeulen 2006).

Current treatment methods in SBDNL involve removal crews treating directionally from the southwest to northeast. As mentioned earlier, *G. paniculata*'s primary means of dispersal is wind; it was thought that the wind's general direction off the lake is consistently to the northeast (assessment of wind data from the Frankfort airport does not support this pattern). For that reason, crews historically have moved from the southwest to the northeast in an effort to prevent tumbleweeds from reintroducing seeds to treated areas (TNC 2013). Manual removal consists of using a spade to sever the plant below the caudex. At SBDNL, cut plant tops are lifted from the

soil and dropped haphazardly on site. At Zetterberg Preserve, cut tops are placed in piles, and although regrowth from these tops has not been observed, abundant seedlings are present around the piles (Howard pers. comm.). This pattern can be attributed to manual removal occurring after seeds have become viable, with mature seeds falling to the soil from the cut tops after they have been placed in the pile. Another concern is the remaining cut tops; several studies have looked at plant regeneration through cuttings from the stems and leaves of *G. paniculata*. When placed in growth medium, 100% of the stem sample explants and 67% of leaf explants formed shoots (Ahroni et al. 1997; Zuker et al. 1997). The likelihood of cut tops establishing roots and overwintering seems low, but this possibility has not been investigated. A final source of regrowth could be the severed taproots that remain in the ground. The taproots are large storage organs that hold massive amounts of energy. Previous assessments of potential resprouting from taproots have been based on community changes in experimental plots (Loope and Siterlet 2000; Emery et al. 2013); no study to date has tracked the fate of individual cut plants. While resprouting from root tissue is perhaps less common than resprouting from stem tissue, propagation from root cuttings is a common practice in horticulture. Moreover, *Silene vulgaris*, from the same family (Caryophyllaceae) as *G. paniculata*, is known to be able to resprout from root tissue (Alberta Invasive Species Council 2014).

Herbicide treatment. In 2013, TNC and NPS started using foliar application of 23.4 ml L⁻¹ aqueous solution of glyphosate (approximately 2% glyphosate 4.5 lbs a.e. per gallon, Roundup ProMax®, Monsanto) as their main treatment method and using manual removal only during poor weather (windy and/or raining) and in remote areas (Howard pers. comm.). Glyphosate is a nonselective systemic herbicide that is readily translocated to areas of the plant with high meristematic activity (Monsanto, Piotrowicz-cieślak et al. 2010). Glyphosate acts by

inhibiting the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase enzyme, which leads to the impairment of protein synthesis (Dyer 1994). Its effectiveness, however, varies with species and timing of application. The experiments which determined that application of 2% glyphosate is most effective have not been made public by TNC. Interestingly, a previous study by Loop and Siterlet (2000) tested 10% glyphosate and found no reduction in *G. paniculata* cover four years after treatment. Perhaps because of this, TNC's approach is to re-treat a previously treated area several times to combat regrowth. TNC thinks that treating with 2% glyphosate several times is more cost effective, better at reducing cover, and safer for the administrators, and also has fewer environmental consequences than treating once with a higher concentration of glyphosate (Howard pers. comm.). That being said, glyphosate is a non-selective herbicide and is necessarily administered with care to prevent accidental spray-over from damaging the surrounding native plant community.

No large-scale quantitative assessment of the effectiveness of *G. paniculata* management in SBDNL has been conducted to date, but Emery et al. (2013) found that removal methods greatly reduced overall coverage within several 20 m x 50 m study plots. It is clear, however, that regrowth in areas treated by summer field crews at SBDNL remains a major problem. A maintenance level has been reached at Wilderness State Park and Petoskey State Park, requiring only one individual to treat once a year for a few hours (TNC 2013). In all treated areas, other common invasive species (*C. maculosa* and *S. vulgaris*) are also present. Arcadia Preserve, the southern-most area known to be infested with *G. paniculata*, experienced a secondary invasion of *S. vulgaris* following removal. Both species are monitored closely at SBDNL, but so far, no secondary invaders have been identified (TNC 2013).

Extended Methodology

Assessment of Control Methods

Treatment Methods. As stated above in the extended literature review, current treatment methods employed by TNC and SBDNL crews (seven to fifteen members) include foliar application of 23.4 ml L⁻¹ aqueous solution of glyphosate (4.5 lbs. a.e. per gallon, Roundup ProMax®, Monsanto), administered at differing rates according to plant density, and manual removal, when wind speeds exceed 10 mph, rain or high temperatures occur, treating remote areas or in close proximity to threatened *Cirsium pitcheri* (Figure 4.1; TNC 2013). Glyphosate, a non-selective systemic herbicide, is administered with care at this site to prevent accidental spray-over damaging the surrounding native plant community. Manual removal consists of using a spade to sever the taproot below the caudex (where stems meet the root), which is thought to prevent resprouting (TNC 2013). Cut plants are removed from the soil but left on site. Treatment at SBDNL typically begins in early June and continues until early August, by which time plants have seeded. Treatment for the present study concluded in late July.

Figure 4.1. Treatment methods used by The Nature Conservancy and The National Park Service. The top images show a crew member spraying glyphosate on a *G. paniculata* plant. The bottom images is a *G. paniculata* plant being mechanically removed and the severed plant top that is left on site.

Figure 4.2. Point-intercept survey grid over experimental and reference areas.

Point-Intercept Survey. Pre- and post-treatment point-intercept surveys were conducted in large unmarked portions of the areas treated by field crews employed by TNC as a routine part of their summer-long treatment efforts at SBDNL. This is an advantageous method for assessing the efficacy of treatment methods as actually applied by field crews, because crews are administering treatment on a large spatial scale and do not know when they are treating the survey areas. Point-intercept surveys are commonly used to assess treatment efficacy in studies of invasive aquatic plants (Madsen 1999, Conklin and Smith 2005,

Mikulyuk et al. 2010, Parks et al. 2016) but are less common in similar studies of invasive terrestrial plants.

Point-intercept surveys were conducted in 2016, 2017, and 2018 to assess the effectiveness of current treatment methods used in Michigan. We established grids (50-m mesh) on two 15-ha treatment areas (Exp1 and Exp2) and a 16-ha untreated reference area with similar densities of *G. paniculata* and other vegetation (Figure 4.2). The sampling grid was generated with ArcGIS software (ESRI™ 10.2) and entered into hand-held GPS receivers (Magellan ProMark3), which field personnel used to navigate to the unmarked sampling points. The reference area enabled comparison with the treated areas; post-treatment changes (if any) detected in treated areas are not attributable to treatment if they also occur in the reference area. We sampled a total of 182 points, of which 122 were treated (56 receiving two years of treatment and 66 one year of treatment) and 60 were reference points. The number of *G. paniculata* plants

within a 2-m radius around each sampling point was counted and divided by the area of the sampling disk ($4\pi \approx 12.57 \text{ m}^2$) to yield an estimate of local density. In addition, *G. paniculata* was scored as present at a given sampling point if the number of plants counted was greater than 0 and absent otherwise; these presence-absence data were used to assess potential changes in frequency of occurrence. Treatment area Exp1 was established in 2016 and surveyed in 2016, 2017, and 2018; treatment area Exp2 was established in 2017 and surveyed in 2017 and 2018. The 2017 and 2018 surveys were completed in three days at the end of May. Due to a miscommunication with field personnel, the 2016 survey occurred over a 2-week period in June in Exp1 area and in a single day in August in the reference area.

Efficacy of the treatment methods was assessed using the mid-P McNemar test for matched-pairs presence-absence (frequency) data (Fagerland et al. 2013) and a nonparametric bootstrap test for count (density) data (R Core Team 2017). These tests assume that post-treatment changes (density, presence-absence) at different survey points are independent; we assessed this assumption with a spatial autocorrelogram based on Moran's *I* and progressive Bonferroni adjustment for multiple comparisons using a modified version of the `correlog` function in R package `ncf` (Bjornstad 2001).

Timing of Treatment. Large 32-m $\times$ 32-m plots were set up throughout the Exp1 area for treatment (Figure 4.3). There were 48 plots in 24 pairs, with one manual removal and one herbicide plot in each pair so the densities were similar between

Figure 4.3. Paired treatment plots in the Exp1 Area. Early season plots are orange, mid season red and late season blue. Plots with blue dots were treated with herbicide.

treatments. We randomly assigned treatment type within each pair. Each plot pair had a minimum of 30-m of space between neighboring plots. Within each larger treatment plot, one 4-m $\times$ 4-m plot was surveyed for *G. paniculata* density. Locations of these smaller plots were determined by tossing the survey pole into the larger plot. Abundance counts of *G. paniculata* were taken from two weeks before treatment until the day treatment was administered from June through July. The number of *G. paniculata* plants within the survey plot was determined and divided by the area (16 m²) to yield an estimate of local density.

Treatment was monitored from June 14 to July 27 in 2016. The timing of plot treatment was separated into three groups: early-season (treatment between June 13 and June 23, 2016), mid-season (treatment between June 27 and July 7), and late-season (treatment between July 11 and July 27). Plants, aside from seedlings, are similar in size throughout this time but at different stages in their phenology. Our goal was to assign manual removal to 8 plots and herbicide treatment to 8 plots in each group, but several weeks of high afternoon winds in June prevented several of the herbicide plots from being treated until July. As a result, early-season treatment consisted of 10 manual removal and 7 herbicide plots, mid-season of 10 manual removal and 6 herbicide plots, and late-season of 4 manual removal and 11 herbicide plots. Plots were resurveyed in June of 2017 to assess treatment efficacy. We also surveyed 10 4-m $\times$ 4-m plots in the reference area in August of 2016 and June of 2017. We performed a Tukey's HSD test to determine how treatment type and timing jointly influence density change.

Resprout Following Treatment. To assess resprouting of individual plants following treatment, prior to the 2017 plant removal period, we marked 125 plants with a minimum of three stems using small aluminum tags affixed to 61-cm metal rebar stakes (Figure 4.4A). The minimum

stem number aided in the selection of mature plants, which we felt were the ones most likely to resprout following treatment and therefore the most sensitive indicator of potential treatment ineffectiveness. The stake was placed in the ground at the base of each plant. Plants were checked for health a week after marking to be sure that the rebar did not damage the taproot when placed in the ground. Any plant that showed signs of declining health (5 individuals) was unmarked and removed from the experiment and another plant located within 1-m was marked. Of these 125 plants, 50 were removed manually, 50 were treated with herbicide, and 25 were left untreated in the reference area (Figure 4.4C). Treatment type was assigned randomly to plants. All treated plants were sprayed or manually removed during one week in early June 2017 to maximize the potential for resprout during the growing season. Marked plants were placed within 2-m $\times$ 2-m plots which received manual or herbicide treatment for uniformity; the plots served to prevent drift of herbicide onto manually treated plants. For additional protection, the marked, manually removed plants were placed under a protective dome while exterior areas were treated with herbicide (Figure 4.4B). The condition of the root crown or upper taproot of the treated plants was checked in late fall 2017 and spring of 2018 to determine whether any resprouting occurred. Plants were located using a Trimble Geo 7x and metal detector.

Data consisted of numbers of root crowns that did and did not resprout in each treatment group. We analyzed the data using a large-sample test for the difference between two proportions (Hollander et al. 2015, p. 495-500) with the Newcombe Hybrid Score confidence interval to compare (1) removal methods and (2) each removal method to the reference area.

Figure 4.4. A: Image showing a marked plant with rebar stake. B: The Nature Conservancy crews spraying herbicide in the area between marked plants; manually removed plants are covered with protective trays. C: Map of marked plants (green: manually removed, orange: herbicide treatment) within the larger Exp2 study area.

Associated Physical Environmental Variables. To assess the relationship between *G. paniculata* presence and selected physical variables, we collected six composite soil samples from infested areas exceeding 25% cover and six soil samples from non-infested areas within our 46-ha study site. Each composite soil sample contained 12 subsamples from a 6-m $\times$ 4-m area and 10-cm depth (Figure 4.5). Michigan State University Soil and Plant Nutrient Laboratory analyzed the samples for grain size and nutrient composition. The soil was classified into eight particle size categories which included very coarse sand (VCoS, 1 mm), coarse sand (CoS, 0.5 mm), medium sand (MS, 0.25 mm), fine sand (FS, 0.15 mm), fine sand-fine fraction (FSFI, 0.1 mm), very fine

sand (VFS, 0.05 mm), silt and clay. Nutrient analysis included pH, total nitrogen (N) and organic matter. To test the soil grain size and nutrient composition differences between where *G. paniculata* was absent and present, we conducted a nonparametric permutation test. Principal Component Analysis was used to determine correlations in the nutrient analysis based on group (present and absent) to visually assess soil nutrient preferences that may be driving *G. paniculata* spread.

The results from the soil nutrient analysis were uninformative. We found that there were no associations or distinctions between nutrients or particle size distribution when *G. paniculata* was absent or present from SBDNL. It seems that *G. paniculata* spread is not limited by the environmental variables we investigated but, in the dunes, it prefers areas with available habitat and limited plant competition. In areas where there is a well-established dense and stable plant community with ground cover exceeding 75% *G. paniculata* is rarely present (personal obs.), this tendency has also been seen in other areas where *G. paniculata* invades (Rudzite 2002).

Figure 4.5. Left: Soil samples were taken from the 12 blue dots at each site and mixed. Right: Soil collections in the field.

Phenology of Seed Maturation

Study Sites and Seed Collection. All seeds were taken from a 40-ha area in the plateau region of Sleeping Bear Dunes National Lakeshore in northwest Michigan, USA (44.872°N, 86.057°W). This area has a *G. paniculata* cover ranging from 25-50% (unpub. data) with no history of prior management. Temperature and precipitation were consistent across years, falling within historical ranges (Frankfort 2NE monitoring station, NCDC NOAA).

For the 2016 collection, seeds were harvested on five dates between July 22 and August 23 from 16 untreated plants within a 20-ha area to determine the phenology of seed maturation. Seeds were also collected on August 23 from 38 plants that had been treated via foliar application of 23.4 ml L⁻¹ glyphosate (Roundup ProMax®, Monsanto) on three different dates between July 11 and July 25, 2016 (one treatment per plant; Figure 6A). In 2017, to avoid collecting from previously treated plants, seeds were collected within a 20-ha area 0.05 km north of the 2016 collection site with similar topography and plant community. Seeds were collected on five dates between July 26 and August 22 from 20 untreated plants (Figure 6B).

Seeds on plants from which collections were taken were abundant but not quantified. Seeds collected from each plant on each collection date were placed in separate paper key envelopes and stored in the dark from 21°C to 23°C in sealed bags containing silica gel beads until the germination experiments were run.

Figure 4.6. A: Image of TNC crew members spraying herbicide on *G. paniculata* plants following flowering. B: Image of mature seed pods which contain up to four seeds per fruit.

Germination Experiments. To ensure that all seeds tested could be checked for germination (radicle emergence) daily within a three-hour window, it was necessary to restrict the germination tests to a subset of the total number of seeds collected each year. A subset of seed samples was randomly selected for the experiment. Specifically, seeds from five of the 16 untreated parental plants from 2016 were used for the seed maturation experiment, while seeds from 12 of the 38 treated parental plants were used for the treatment-date experiment. Seeds from 12 of the 20 untreated parent plants from the 2017 collection were used. In all germination experiments, 100 seeds collected from each parental plant on each collection date were placed on moist filter paper (MN 410) in 94-mm $\times$ 16-mm Petri dishes and incubated in a growth chamber at 20°C and 112 $\mu\text{mol m}^{-2}\text{s}^{-1}$ photosynthetically active radiation from florescent bulbs with a 12:12 h light:dark photoperiod (Figure 7A). A total of 2,500 seeds were tested from the 2016 collection (100 seeds/plant $\times$ 5 plants/collection date $\times$ 5 collection dates) and a total of 6,000 seeds from the 2017 collection (100 seeds/plant $\times$ 12 plants/collection date $\times$ 5 collection dates). Each day for 12 days, the same individual checked the Petri dishes (to minimize bias) and newly germinated seeds were counted and removed. Germination was operationally defined as radicle emergence (Baskin & Baskin 2001; Figure 7B-D). Petri dish positions in the growth chamber were randomized daily to avoid potential biases in temperature or light exposure. To show that experimental conditions were adequate, commercial seeds known to have germination percentages in excess of 90% were also tested. The commercial seeds used were of the Early Snowball cultivar distributed by W. Atlee Burpee & Co and were produced in the Netherlands in 2016. The same procedure was employed in the glyphosate treatment date experiment except that each parental plant was represented by a single set of 100 seeds with the same treatment date and

same collection date; a total of 1,200 seeds were tested (100 seeds/plant $\times$ 4 plants/treatment date $\times$ 3 treatment dates).

Figure 4.7. Top A: Seeds in Petri dishes in the incubator. B-D: Images of germinated *G. paniculata* seeds. Seeds have snail like shape, can see the white radicle emerging and some root hairs in image C.

Statistical Analysis. For each collection date of untreated plants and glyphosate treatment date of treated plants, the temporal pattern of germination during the 12-day experiment was characterized by fitting a nonparametric Kaplan-Meier time-to-event curve (McNair et al. 2012). Germination maturation patterns for different collection dates were compared pairwise using log-rank tests with Holm correction for multiple comparisons, as were germination maturation patterns for different glyphosate treatment dates (McNair et al. 2012). All statistical analyses were performed using R software, version 3.4.1 (R Core Team 2017).

Conclusions and Implications

Many questions remain unanswered regarding the impact of *G. paniculata* in its introduced habitat and the best removal methods to employ. It is evident that *G. paniculata* is now the dominant plant species found on infested sections of the dunes. Consequently, addressing these questions is now a priority in order to preserve the unique dune community. My research will address some of the identified gaps in the literature to better understand introduced *G. paniculata* and to assess the effectiveness of currently used treatment methods. This research, combined with the contributions of other researchers, will culminate in an adaptive management plan specific to *G. paniculata* in northwest Michigan. While the topics in this thesis may seem only loosely related, they are all integral parts of the adaptive management plan. With this plan, managers in northwest Michigan will have the tools to more effectively control infestations while optimizing their resources. *G. paniculata* is invasive not only in Michigan but in many areas throughout North America. The management plan developed in this project can be adapted by organizations elsewhere and can also be altered to other invasive species in northwest Michigan dune habitats, such as *S. vulgaris*. The ultimate outcome of this research is that the implementation of the adaptive management plan will lead to the control of invasive *G. paniculata* and protection of the many unique native species found in the dunes of northwest Michigan.

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Appendices

Appendix 1: Supplemental Material Chapter 2

Table S2.1. Presence-absence data from the point-intercept survey.

	Absent- Absent	Present- Absent	Absent- Present	Present- Present	Total
Exp1 2016-2017	12	2	1	41	56
Exp1 2016-2018	12	4	1	39	56
Exp2 2017-2018	9	11	0	46	66
Ref 2016-2017	9	2	3	46	60
Ref 2016-2018	7	2	5	46	60

Appendix 2: Supplemental Material Chapter 3

Table S3.1. Summary of climate information in 2016, 2017 and historical averages from Frankfort 2NE monitoring station located 27 km south of the study site (MRCC 2018).

Year	Mean Temp (°C)	Min Temp (°C)	Max Temp (°C)	Precipitation (in)
2016	10	6	14	32
2017	9	5	12	33
1998-2017*	9 (7-10)	5 (4-6)	13 (11-14)	32 20-41)

*The average historical ranges include the maximum and maximum climate information within parenthesis.

Table S3.2. Holm-corrected P values for pairwise comparisons of the germination patterns whose Kaplan-Meier time-to-event curves are shown in Figure 1A Dates are the seed collection days in 2016.

	Jul 22	Jul 28	Aug 4	Aug 12
Jul 28	< 0.0001	–	–	–
Aug 4	< 0.0001	< 0.0001	–	–
Aug 12	< 0.0001	< 0.0001	< 0.0001	–
Aug 23	< 0.0001	< 0.0001	< 0.0001	0.7097

Table S3.3. Holm-corrected P values for pairwise comparisons of the germination patterns whose Kaplan-Meier time-to-event curves are shown in Figure 1B. Dates are the seed collection days in 2017.

	Jul 26	Aug 1	Aug 8	Aug 16
Aug 1	< 0.0001	–	–	–
Aug 8	< 0.0001	< 0.0001	–	–
Aug 16	< 0.0001	< 0.0001	< 0.0001	–
Aug 22	< 0.0001	< 0.0001	< 0.0001	0.093

Table S3.4. Holm-corrected P values for pairwise comparisons of the germination patterns whose Kaplan-Meier time-to-event curves are shown in Figure 3. Dates are the days on which source plants were treated with glyphosate.

	Jul 11	Jul 18	Jul 25
Jul 11	–	–	–
Jul 18	< 0.0001	–	–
Jul 25	< 0.0001	0.0077	–

Appendix 3: Supplemental Material Chapter 4

Table S4.1. Soil nutrient concentrations when *G. paniculata* is absent (A) and present (P) from study areas Exp1 and Exp2.

Name	pH	P (ppm)	K (ppm)	Mg (ppm)	Ca (ppm)	CEC*	K (%)	Mg (%)	Ca (%)	OM (%)	NO ₃ ⁻ (ppm)	NH ₄ ⁺ (ppm)
1A	8.8	15	11	25	427	2.4	1.2	8.8	90.0	0.1	0.8	1.2
2A	7.8	15	72	210	1131	7.6	2.4	23.1	74.5	2.6	0.6	6.6
3A	8.3	10	32	88	907	5.4	1.5	13.7	84.8	1.2	2.0	4.3
4A	8.1	6	44	113	742	4.8	2.4	19.8	77.9	1.2	0.6	3.4
5A	8.1	8	28	108	615	4.0	1.8	22.2	76.0	1.4	1.9	3.7
6A	8.9	11	10	35	433	2.5	1.0	11.7	87.2	0.1	0.2	1.6
1P	8.1	12	19	83	545	3.5	1.4	20.0	78.6	0.8	2.9	2.7
2P	8.3	11	25	92	613	3.9	1.6	19.7	78.7	0.9	1.3	3.8
3P	8.4	9	19	49	448	2.7	1.8	15.1	83.1	0.4	2.0	2.3
4P	8.3	5	21	56	394	2.5	2.2	18.7	79.1	0.5	1.6	1.8
5P	8.5	5	11	43	321	2.0	1.4	18.0	80.6	0.3	1.5	1.7
6P	8.4	6	19	42	359	2.2	2.2	16.0	81.8	0.4	1.3	1.6

*CEC is measured in (meq/100g)

Appendix 4: Lessons Learned

I have included a lessons learned section because, as the title implies, I learned many valuable lessons during this research and would like to aid others doing similar work from having to learn these same lessons. To dive right in, the Timing of Treatment plots were 32-m x 32-m and 48 were treated in total. We thought this would facilitate a more accurate change in density number because the crew would treat the area as they normally would due to the large size and number of plots. In reality, however, it seemed to be a bit demoralizing for the crew, especially having to backtrack and treat the buffer strips between plots. While *G. paniculata* removal crew normally treat large areas and have a goal in mind, it was somewhat less intimidating for them to see the unmarked area stretching before them every day. In the future I would divide the area into three strips for early, mid and late season treatment, and then divide each strip into four sections, two for manual removal and two for herbicide application (to account for differences in topography). With this setup, the crew could treat in their typical transect fashion with no buffer spaces between plots. One would simply sample 5-m within each section to avoid areas that may have been exposed to spray over.

Another valuable lesson learned during this project was that not to send soil samples to general agricultural extension offices for analysis. I found that the analysis is not in as fine of a scale that was required for this project and for that reason, the grouping that resulted from our Principal Component Analysis was suspect. As a result, soil analysis was omitted from my thesis manuscript chapters.

Another change I would make if given the opportunity to do this research again is to alter my modified Braun-Blanquet scale (with 0 = absent, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 =

76-100%), to a finer scale that is more informative. However, by making that change, the data would be more subjective. It is a tradeoff to consider, but in this case the data would have been more informative with a finer scale because most plots fell in category 1 or 2 even when they had vastly different *G. paniculata* counts. This was particularly true with surveys following treatment when most plants have small above ground biomass and were mostly comprised of seedlings and immature plants. I was not able to use the vegetation cover data for this reason and found the density counts to be a more informative comparison of pre and post treatment analysis.

If I were to continue this project in the future I would also measure sand movement following manual removal versus sand movement when herbicide or no treatment is used. We found that the rebar marking the plants monitored for resprouting were typically buried when treated manually but just under the surface when herbicide was used. The soil hardness and composition also played a role, but it would be interesting to investigate how disruptive manual removal is in the dunes. Another question that would be easy to answer in the future is whether or not the manually removed cut tops have the ability to survive over the winter. From what I have seen on the dunes it is unlikely, but we did observe removed plant tops putting out lateral roots the fall after treatment. While it is unlikely the plant tops would survive over winter, a formal study would answer that question with certainty and be fairly simple to execute.

Another aspect of the study that would be very interesting is long term monitoring of the plant community changes following treatment. The dunes do not have a long-lived seed bank due to the harsh conditions, so the changes will occur slowly. This is particularly true in the case of *C. pitcheri*, a native species of interest, which takes four to eight years to mature and produce seeds before dying. To get an accurate analysis of treatment impacts, dune plant community surveying would need to continue for at least 10 years (preferably more).

Hopefully these lessons learned will be valuable for others in the future who are conducting research in the dunes or a project with similar methods. I have had an incredible time over the last two years conducting this research and am happy to be able to pass along this information to the next researcher fortunate enough to work in this incredibly beautiful and dynamic Great Lakes dune ecosystem.

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