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# The Effect of Site Characteristics on the Reproductive Output of Lesser Celandine (*Ranunculus ficaria*)

Justin P. Kermack  
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THE EFFECT OF SITE CHARACTERISTICS ON THE REPRODUCTIVE OUTPUT  
OF LESSER CELANDINE (*RANUNCULUS FICARIA*)

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Bachelor of Science in Environmental Science

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May 2014

submitted in partial fulfillment of requirements for the degree

MASTER OF SCIENCE IN ENVIRONMENTAL SCIENCE

at the

CLEVELAND STATE UNIVERSITY

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**ABSTRACT**

*Ranunculus ficaria* L., an ephemeral perennial invasive plant brought over from Europe, is becoming widespread throughout the Northeastern United States. This herbaceous buttercup is able to create extensive dense mats that limit native species growth. Taking advantage of an early growing season and rapid reproduction rates, this species can create dense monocultures, which threatens native communities and ecosystems. Elimination of native spring ephemerals results in decreased biodiversity. A better understanding of how *R. ficaria* responds to site characteristics is needed to prioritize management efforts toward high-risk sites.

*Ranunculus ficaria* abundance and reproductive output (seed, bulbil and tuber production rates) were examined in plots spanning a disturbance gradient away from a river. Site characteristics (PAR, aspect, soil pH, soil moisture, texture and nutrient content) were investigated to examine their role in plant performance. I hypothesized that soil characteristics (pH and nutrient availability) drive *R. ficaria* plant performance; specifically I expected higher biomass and reproductive output to be associated with higher soil pH. I also expected reproductive output and *R. ficaria* biomass would be highest in moist floodplain at intermediate distances from rivers.

Many soil nutrients and characteristics were significantly related to biomass and reproductive output; specifically phosphorus, calcium and LTI (Lime Test Index) all showed significantly positive relationships with plant biomass and bulbil counts, while

soil pH was significantly positively related to biomass. Bulbil and tuber counts were significantly higher in soils of high percent silt. These findings suggest that soil characteristics (pH, texture) and nutrients (P, Ca) are strongly linked to plant performance, supporting my hypothesis. Reproductive output and *R. ficaria* biomass were not significantly greater at intermediate distances from rivers, in contrast to my hypothesis.

A plant performance model was generated using object-based image analysis with the aim of creating an accurate classification of sites in terms of suitability for *R. ficaria* performance. A large scale field survey was used to assess model predictions, which were found to be 68 % accurate. Overall, this study was able to expand on the current limited understanding of *R. ficaria*, which can prove helpful in aiding management to reduce population size and spread.

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## CHAPTER 1

### INTRODUCTION TO INVASION

#### 1.1 INTRODUCTION

The invasion of alien species is a global dilemma, as they threaten to damage ecosystems and replace native communities (Jose et al., 2013). Invasive plants are non-native plants that have harmful consequences, which can lead to a myriad of undesired economic and environmental impacts. Economic and ecological consequences have been well documented by many ecologists over many decades (Elton, 1958; Pimentel et al., 2005; Whitfeld et al., 2014). Invasive plants can alter ecosystem functioning, threaten native species' populations, reduce biodiversity, degrade the environment, cause economic loss and may cause physical harm to humans (Jose et al., 2013). The total worldwide estimated economic damage caused by invasive species is over \$1.4 trillion, according to a report conducted in 2012 by the Pacific Northwest Economic Region (PNWER) Invasive Species Working Group. The annual economic costs to the U.S. caused by invasive species have been estimated to be as high as \$120 billion (Pimentel et

al., 2005). Major damages can be attributed to crop weeds, insect pests, plant pathogens, livestock diseases, microbes and parasites (Jose et al., 2014; PNWER, 2012).

Through the proliferation of human migration and trade, pathways for invasive aliens to invade regions beyond their native boundaries continue to increase (Dukes and Ziska, 2014). This important link between increasing global trade networks with increased biological invasion has been documented by Kaluza et al. (2010). Sea transportation accounts for 90 percent of world trade and is largely hidden from the public eye and underexplored as a mechanism for invasion (Kaluza et al., 2010). Many unintended invasive problems were set in motion before our current understanding of invasion pathways and consequences were well-developed. To this day, many non-native plants that were introduced as a result of horticulture trade continue to result in invasion problems (Bradley et al., 2012). As we better understand the underlying mechanisms of invasion and its consequences, we are better able to detect threats and protect native ecosystems.

Early recognition and management of potentially harmful species is incredibly important to effectively control an invasion. The longer an invasion persists, the more difficult and costly it is to manage, requiring increasing amounts of resources and effort (Hobbs and Humphries, 1995, Figure 1-1). Land managers are also limited by their available resources and scientific knowledge to appropriately control harmful infestations (Jose et al., 2014). Additional exacerbating factors contributing to the current invasive dilemma are policy deficiencies, a lacking social organization to contest these invasions and a general lack of public awareness (Simberloff et al., 2005; Miller; Schelhas, 2009). Due to limited resources, more realistic management goals should aim to prioritize

control and focus on managing populations to levels that do not adversely impact the integrity of the ecosystem (Jose et al., 2014).

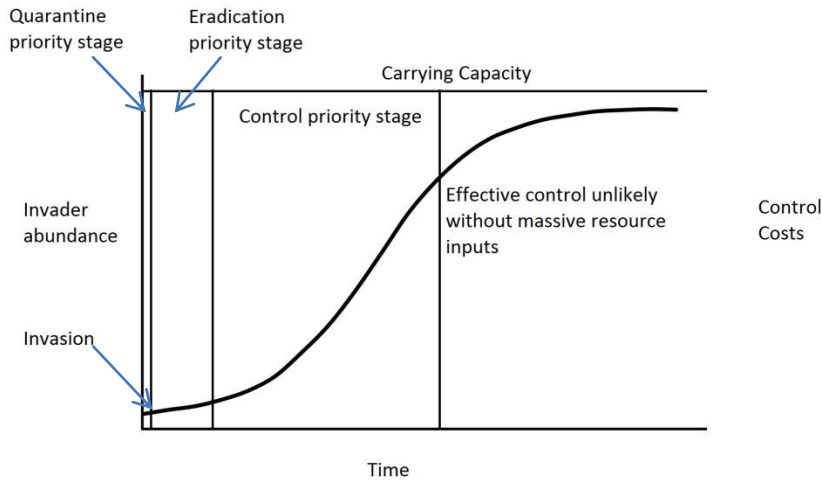


Figure 1-1: The phases of weed invasions along with priorities for each stage. The more time that passes after an invasion occurs, the more difficult and costly it is to control (redrawn from Hobbs and Humphries, 1995).

Invasive plants display a number of physiological traits that make them ideal candidates for success in introduced regions. The proverbial “Darwinian demon” (Law, 1979) has been used to describe a hypothetical organism, which exhibits maximized fitness of all aspects under unconstrained evolution. Some characteristics include high survival in any environment and high reproduction rates, occurring both often and early in any environment (Law, 1979). This idea has been used to try to understand life history strategies of organisms and many invasive plant species display similar traits to some degree. In a similar vein, Baker (1965, 1974) compiled a list of ideal weed characteristics, which has been used as a tool to help identify which weeds will become invasive (Chaney and Baucom, 2012). While there is no single trait that can consistently predict the successfulness of a species, it can be useful for management purposes to have an idea

of what successful invasive plant characteristics would include, as this could help shift priorities and resources towards species of greater invasion risk.

Not all alien species end up causing harmful effects in a newly invaded region; the risks are species and community dependent. Williamson (1993) coined the “tens rule”, which estimates that one in every ten imported species will escape, one in ten introduced species will become established and one in ten established species will go on to become a pest. It has been claimed that the actual chance of invasion success is actually much higher, with estimates closer to 50% (Jeschke and Stayer, 2005). The challenge is to identify the specific introduced species that will likely be detrimental to a particular region, in order to prioritize management and control efforts (Jose et al., 2013). Not only is it important to identify the most risky species, the time of intervention is also extremely important.

There are numerous invasion hypotheses that try to explain invasions through focusing on either ecosystems, invaders or a combination. Metanalyses show high variability of empirical support for some of the various invasion hypotheses such as novel weapons, biotic resistance, enemy release, the tens-rule and invasion meltdown, among others (Jeschke et al., 2012). The three hypotheses with the greatest level of support all consider invader-ecosystem interactions, which include invasional meltdown, novel weapons and enemy release, respectively. Invasional meltdown refers to the process where one invasive species establishment can facilitate the invasion path for other non-native species (Simberloff and Von Holle, 1999). The novel weapons hypothesis proposes that certain invasive species may possess allelopathic, defense or antimicrobial chemicals, making the species advantageous over its native competitors (Callaway and Ridenour,

2004). The enemy release hypothesis states that there will be lower levels of herbivory experienced by a species in its introduced range due to the escape of its naturally adapted enemies in its native range resulting in increased distribution and abundance (Keane and Crawley, 2002). These three hypotheses, among others such as the “tens rule”, add to our collective knowledge of invasion mechanisms and grant ecologists useful conceptual models that can be applied to help continue filling the gaps in the scientific literature of invasive ecology. Additional factors such as disturbance levels, influence invasion success and will be explored in this study. Disturbances, such as flooding events, result in loss of vegetative cover, which frees up space and resources for invaders to spread into new areas (Catford et al., 2012). The consequences of invasion can be exacerbated if the invasive species has a high reproductive output, displays rapid lateral growth and if it blooms before competitors have a chance to capitalize on the newly available resources (Catford et al., 2012).

While there are many hypotheses that attempt to explain invasion success, there are many factors that can cause invasion attempts to be unsuccessful, such as biotic resistance and invasional interference. Biotic resistance relates to the ability of native species to decrease the success of exotic invasions in a community (Levine et al., 2004). While biotic resistance rarely repels invasions completely, there is evidence for constraint of invasive species abundance after establishment (Levine et al., 2004). Invasional interference occurs when invader-invader interactions are subadditive, reducing the invasion success for each invader (Rauschert and Shea, 2012; Yang et al., 2011). When invasional interference is observed and a congener is present, it is unlikely that there will be a change in the net effect (Rauschert and Shea, 2012). For effective management, it is

important to understand and identify these various interactions so that limited resource allocation can be effectively prioritized to target strategically key invasive populations, resulting in the most positive net change.

Specific invasive species will thrive in areas that are most conducive to their specific environmental requirements and preferences, such as climatic conditions. Some regions however, are more susceptible to invasion in general than others. For example, roads have strong associations with invasion as they provide a means for long distance transportation (Mortensen et al., 2009). Ohio is extremely susceptible to invasions due to the extensive transportation network from roads to waterways. Ohio has more road miles than any other Midwest state, making it a hub for commerce, people, and invasive species (OIPC, 2015). The temperate climate also provides alluring growing conditions for many invasive species, along with sufficiently regular disturbances for invasive plant establishment.

Invasive plant species are not always invasive in all ecosystems and there is variation in invasiveness across the established environments (Rew et al., 2005). There are many environments where notoriously invasive plant species go extinct locally preceding rare establishment. This implies that focused management should consider management based on populations segregated by environment, rather than across their entire distribution range (Rew et al., 2005). Management should also prioritize environments where an invasive is expected to perform well.

The Ohio Department of Agriculture (ODA) developed the noxious weed list for Ohio, which currently includes 19 harmful species. Some of the most damaging invasive



plants in Ohio according to the Ohio Department of Natural Resources (DNR) include, Japanese knotweed (*Polygonum cuspidatum*), purple loosestrife (*Lythrum salicaria*), phragmites (*Phragmites australis*), multiflora rose (*Rosa multiflora*) and Japanese honeysuckle (*Lonicera japonica*), among others. Lesser celandine (*Ranunculus ficaria* L.) is another high priority invasive of increasing concern, which is on the candidate list for inclusion in the new ODA list of banned species. This new invader is poorly understood, therefore several OIPC research priorities address the performance of this species. *R. ficaria* is the species of interest for this research project. It is vitally important to gain a greater understanding of the environments in which *R. ficaria* thrives and will become problematic, as well as identifying the invader mechanisms influencing the population dynamics of this invasive species. The Cleveland Metroparks has listed *R. ficaria* as one of its top management priorities due to its impact on the Rocky River watershed. Flooding events and river systems appear to play an integral role in transporting propagules of this herbaceous perennial. If spread continues at this rapid rate, it will cause exceedingly detrimental effects to the spring flora in the Rocky River area and beyond, making effective management essential. Gaining a greater understanding of seed production is also a primary proposed research question of the OIPC (OIPC 2016, <http://www.oipc.info/help-answer-research-questions.html>).

*Ranunculus ficaria* is a major species of concern to land managers in Ohio. It is found throughout Ohio, in sites of differing characteristics, provided there are sufficient moisture levels (Axtell et al., 2010). In the Cleveland area, there is a large established infestation around the floodplains along the Rocky River. Over 183 acres of the reserve have been identified as having more than fifty percent *R. ficaria* cover. Estimates on

actual acreage were as high as three hundred acres or more in 2008 in the Rocky River Reservation (Mack, 2008). River valleys and floodplains have the highest infestations and commonly contain complete monocultures established at extremely high densities.

According to Metropark managers, the origin of the infestation seems to have come from a house just north of Cedar Point Road, where it may have been planted as an ornamental. The entire slope below this house is completely covered with the invasive buttercup, with an unusually high cover of *R. ficaria* on such a steep gradient. The Rocky River flows north towards Lake Erie, and it is interesting to note that *R. ficaria* populations are extremely low southward from the hypothesized origin. The *R. ficaria* populations north of this particular house are extremely dense and healthy, especially in lowland floodplains along the Rocky River banks. This supports the idea that flooding events and river systems play an important role in explaining the spread of this invasive species through transportation and deposition of propagules.

There are five known subspecies of *Ranunculus ficaria* L present in the United States, namely: subsp. *ficariiformis*, subsp. *chrysocephalus*, subsp. *calthifolius*, subsp. *ficaria* and subsp. *bulbifer* (Sell, 1994). The subspecies *calthifolius* and *ficaria* are diploid, while all other subspecies of *R. ficaria* are tetraploid. Diploid subspecies do not produce bulbils (Marsden-Jones, 1933). Using distinguishing characteristics provided by Axtell et al (2010) and Sell (1994) it was determined that Rocky River consists primarily of subspecies *bulbifer*, with some spotty occurrence of subspecies *ficariiformis*. The subsp. *bulbifer* is characterized by having bulbils present in the leaf axils and more elongated stems with less leaves crowded at the base (Axtell et al., 2010). The larger, less common subsp. *ficariiformis* is characterized as twice the size of the subsp. *bulbifer*, with robust

stems and bulbils also present (Axtell et al., 2010). All collections made in Chapter 2 and 3 were subsp. *bulbifer*.

Many invasive plants in North America are known to have an early growing season compared to other natives (Wolkovich and Cleland, 2010). *R. ficaria* is a good example of this phenomenon, which flowers earlier in spring than native plant species (Swearingen, 2005). Through this mechanism, *R. ficaria* is able to establish early before the tree canopies grow back and block the sunlight from penetrating. *R. ficaria* ends its growing season early, which is unusual for invasive species. Another alarming characteristic of *R. ficaria* is its ability to form extensive mats that, in conjunction with its early growing season, allow it to outcompete its native competitors (Axtell et al., 2010). This can lead to complete monocultures in some cases, which decreases the biodiversity of an area. During senescence around late June, large portions of land are left barren, making them susceptible to invasion by a host of weedy species (Axtell et al., 2010).

Another possible mechanism of invasion success is the use of “novel weapons” such as the secretion of toxic chemicals (Callaway and Ridenour, 2004). *R. ficaria* is potentially allelopathic, although an allelochemical has not been identified and there have been limited experiments testing this theory (Cipollini et al., 2012). *Ranunculus ficaria* contains an array of chemical compounds such as ascorbate, tannins, protoanemonin and anemonin (Chevalier, 1996). Although their impact on other plants is largely unknown, protoanemonin, which is present in fresh leaves, is toxic to most mammals, and while seldom fatal, ingestion can cause sickness (Taylor and Markham, 1978). Dry leaves however, do not contain this harmful compound, as the protoanemonin is not present (Axtell et al., 2010).

One of the paramount difficulties facing the management of *R. ficaria* is the lack of scientific information about its basic biology and demography; it is a difficult task to manage a species that is not fully understood. Chapter 2 of this research project investigates the relationship of site characteristics to *R. ficaria* plant performance. One of the goals of this study is to identify what site characteristics favor *R. ficaria* growth through quantifying seed, tuber and bulbil production and germination in a variety of environments. Through studying the reproductive output of *R. ficaria* under these varying disturbance gradients, soil composition, topography and slope face direction, I aim to gain insight into seed, bulbil and tuber production rates and note the degree to which varying site characteristics influence the productivity of the species.

Land managers need to know where to focus their limited management efforts when dealing with plant invasions. Geographical Information Systems (GIS) and Remote Sensing (RS) programs can be powerful tools in the arsenal of invasive plant managers, and the number of publications making use of these tools has rapidly increased (Joshi et al., 2004; Singh et al., 2015). There are many cost-effective methods of modeling, which can provide accurate field representations that help to prioritize allocation of limited resources for managing populations of concern. Modeling approaches have been used with variable success in ecology, with many approaches producing accurate and useful results (Ghioca-Robrecht et al., 2008; Michez et al., 2016; Van Lier et al., 2009). The most common GIS approaches include the production of maps investigating actual invader distribution, plant performance, potential distribution and areas at risk of invasion (Joshi et al., 2004). There are many well-documented benefits encouraging the use of GIS and RS tools, such as the ability to study inaccessible ecosystems, complex terrain types,

multispectral and multitemporal data in a cost effective manner (Joshi et al., 2004). Studies can vary substantially in success, depending on the species of interest, imagery sources and resolutions, and differences in methodologies and expertise (Joshi et al., 2004). Despite the alluring benefit of being able to remotely survey difficult-to-access areas, in many cases more information and fieldwork are necessary to produce and assess the accuracy of models. Separate data collections, which are not used to inform model predictions, are necessary to assess the accuracy of actual occurrence and plant performance maps without bias. Reproductive information is also necessary to inform demographic models (Jongejans et al., 2008).

Chapter 2 involves a two-year observational study aimed at understanding the influence of site characteristics on *R. ficaria* performance. Chapter 3 includes a GIS-based plant performance model of *R. ficaria* that aims to predict the successfulness of this invasive based upon a variety of site characteristics. A separate survey was conducted to provide a ground-truthing dataset for the model. An understanding of influential site characteristics, along with basic information such as reproductive outputs and biomass measurements, were necessary in generating and analyzing the effectiveness of the model. Chapter 4 summarizes the main findings of this project. Overall, the project aims to advance the understanding of population dynamics and drivers involved with the invasive perennial, *R. ficaria*.

## CHAPTER II

### OBSERVATIONAL STUDY OF SITE CHARACTERISTICS EFFECT ON THE REPRODUCTIVE OUTPUT OF LESSER CELANDINE (*RANUNCULUS FICARIA* L.)

#### 2.1 ABSTRACT

*Ranunculus ficaria*, an ephemeral perennial invasive plant brought over from Europe, is becoming widespread throughout the Northeastern United States. There is cause for concern as its high production of bulbils and tubers, linked with its ephemeral growth pattern, allow *R. ficaria* to outcompete, disperse and establish more rapidly than its local competitors. Elimination of native spring ephemerals results in decreased biodiversity.

*Ranunculus ficaria* abundance and reproductive output (seed, bulbil and tuber production rates) were examined in plants collected from plots spanning a disturbance gradient away from a river. Site characteristics (PAR, aspect, soil pH, soil moisture, texture and nutrients) were investigated in order to examine their role in plant performance. I

hypothesized soil characteristics (pH and nutrient availability) would drive *R. ficaria* plant performance; specifically I expected higher biomass and reproductive output when pH was higher. I also expected that reproductive output and *R. ficaria* biomass would be highest in moist floodplains at intermediate distances from rivers.

There was high variability observed between sample sites, with bulbil production ranging from 0 to as high as 18 per plant (mean = 2.851) and tuber production ranging from 1 to 11 tubers per plant (mean = 2.417). Densities of *R. ficaria* were found to be as high as 11,425 plants/m<sup>2</sup> in some areas with an overall mean of 2,772 plants/m<sup>2</sup>. No seed production was observed during sampling and very low seed production was observed during field observations the second year, showing near complete reliance on asexual reproduction. Numerous soil nutrients and characteristics were significantly related to biomass and reproductive output; specifically phosphorus, calcium and LTI all showed significantly positive relationships with plant biomass and bulbil counts, while pH was significantly positively related to biomass. Calcium levels were significantly higher in soil of higher percent clay and silt. Soils with high percent silt showed significantly positive relationships with bulbil counts, tuber counts and tuber mass, which may be driven by high calcium levels. These findings imply that soil characteristics (pH, texture) and nutrients (P, Ca) are strongly linked to plant performance, thus I can support my hypothesis. Reproductive output and *R. ficaria* biomass were not significantly greater at intermediate distances from rivers, thus I cannot support my hypothesis. This study was able to expand on the current limited understanding of *R. ficaria*, which can prove helpful in reducing population size and spread.

## 2.2 INTRODUCTION

Invasive plant species will often thrive in a new habitat where they can experience a rapid growth rate along with high reproductive output, causing substantial harm to native communities. Spring ephemeral plant communities are under threat from invasive plant species and are often outcompeted to critically low levels (Axtell et al., 2010). The replacement of native spring ephemerals by a few invasive species results in decreased biodiversity and significant changes to the local ecosystems (Zhang et al., 2007).

Because there aren't enough resources to manage all invasive plants, managers are faced with prioritizing their limited efforts. Invasive plants are not always harmful in all environments and being able to identify the most susceptible environments for invasion is crucial for focused management and control efforts (Roxburgh et al., 2004). Identifying the site variables behind successful invasive growth can help prioritize more problematic invasions.

Disturbance can be a major driving force behind invasion success as invaded ecosystems are rarely free from disturbance and habitat loss (MacDougall and Turkington, 2005). This causes uncertainty as to whether invasive species drive community change or if they are passengers to the process. The “driver” and “passenger” models have been used to describe these processes (MacDougall and Turkington, 2005). Despite competition playing a role, studies have found that the “passenger” model more aptly explains the dominance of invasive species, where environmental conditions are more influential than interactive forces (MacDougall and Turkington, 2005; Davis, 2003).



Sources of disturbance that have more crippling impacts on native species include climate change and altered disturbance regimes (MacDougall and Turkington, 2005). Competition effects still can influence invasional success, yet as many ecological studies have found, the impacts have been far less convincing in comparison to disturbance, intertrophic interactions and habitat loss (Davis, 2003; Levine et al., 2003). While superior competitive ability is often hypothesized as a cause of invasion success, meta-analyses have found that less than 5% of studies (over 150 studies analyzed) tested whether plant invasions were aided via competition between species, alteration of ecosystem variables, allelopathy or other processes (Levine et al., 2003). Additional influences on population dynamics include climate, soil characteristics (pH, nutrients, texture, moisture), light availability, aspect, slope gradient, and local herbivore and plant species present. While there is much ecological interest in invasion population dynamics, there is still much to understand about the mechanisms and pathways that lead to the impacts observed. With greater understanding of these processes relevant to a particular species, resources can be more efficiently managed to most efficiently combat the spread of that invasive.

*Ranunculus ficaria* L., also known as *Ficaria verna* (Sell, 1994), is a herbaceous invasive that was brought over to North America for horticultural purposes (Axtell et al., 2010). It is an alluring garden plant, producing attractive yellow flowers early in spring, and it continues to be sold by nurseries around North America. *Ranunculus ficaria* is native to Europe and parts of Asia, and is mainly found in floodplains and wetland lowlands. It is believed to spread via flooding events as well as being unearthed and transported via human and animal interactions, such as seeds sticking to animal fur or the

shoe of a hiker (Swearingen, 2005). It can also be found in drier, woodier highlands.

This invasive buttercup is becoming increasingly prevalent throughout the northeastern United States, with more recent occurrences noted in southern states such as Texas.

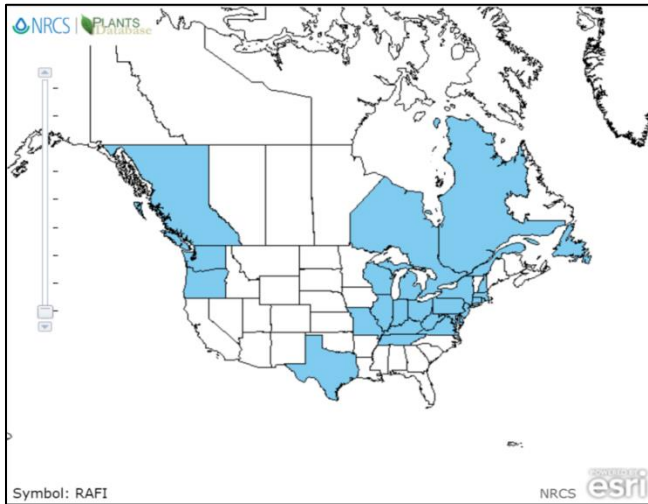


Figure 2-1: The distribution of *R. ficaria* in North America, obtained from the USDA Plants Database. A large portion of the northeastern United States has *R. ficaria* present, as well as geographically diverse areas such as Texas and the Pacific Northwest. Focused management efforts are required to contain the invasion and limit the continued spread of this troublesome invasive.

Invasive plants such as *R. ficaria* often display large variations in reproductive output, which is not ideal for land managers (Jung et al., 2008). Not enough is known about *R. ficaria* reproductive output to understand the population growth of this plant and which environments are most susceptible to invasion. It is imperative to gain a greater understanding of this perennial in order to more efficiently manage and control its spread. This greater understanding is vitally important for land management due to limited funds and resources. Fortunately, it is not well established yet in all areas, allowing the management and prevention of spread if effective methods are identified. It is necessary to gain control over this invasive plant before it becomes too widely established, where management costs and control efforts would increase rapidly.

Showy invasive species such as *R. ficaria* have been known to impact the rates of pollinator visitation to native species, which can lead to a reduction of native plant species (Masters and Emery, 2015). Conversely, the showy flowers can increase pollinator activity and thus aid the pollination of native plants. The influence of *R. ficaria* on pollinator visitation rates and seed production have been investigated using two native species (*Claytonia virginica* and *Cardamine concatenata*), with results indicating significantly greater pollinator activity and higher visitation rates per flower for *Claytonia virginica*, yet seed production was found to be greater in uninvaded habitats for *Cardamine concatenata* (Masters and Emery, 2015). This implies that both the facilitative and competitive effects should be considered during management.

Variable leaf litter depths are common to riparian corridors due to the variation in flooding frequency and intensity (Masters and Emery, 2015). While a lack of litter and deep litter decreased *R. ficaria* biomass, bulbil production was not different across treatments and seed production only decreased in deep litter of 20 cm (Masters and Emery, 2015). This maintenance of reproductive output across a variety of leaf litter depths may provide an advantage over native species competing for resources (Masters and Emery, 2015).

Soil characteristics can also impact plant communities and species performance. Soil pH can influence the availability of plant nutrients considerably and can often lead to limitation of plant growth especially under increasing acidity (Petra and Proctor, 2001). Invasive and native plants can have similar interaction with respect to soil variables (Porazinska et al., 2003). *Alliaria petiolata* (garlic mustard) is an invasive species that thrives in similar environments as *R. ficaria*, preferring shaded moist floodplain forests,

and high levels of disturbance (Byers and Quinn, 1998). While able to grow in very sandy soils, garlic mustard prefers rich soils (Lewis et al, 2006). This plant exhibits a high level of plasticity, which allows it to thrive in a wide range of environmental conditions, making it a successful invasive plant (Rebek and O'Neil, 2006).

The aim of this study was to determine the effect that varying site characteristics would have on the abundance and reproductive output of *R. ficaria* along a disturbance gradient away from a river. To accomplish this, biomass and reproductive output were measured at various sites along the Rocky River, to compare to site characteristics. Reproductive output and plant biomass were expected to be highest in moist floodplains at intermediate distances from rivers, where plants will experience moderate levels of disturbances along with moist yet not fully saturated soils. Soil characteristics (higher pH and nutrient availability) were also hypothesized to play an important role in determining successful *R. ficaria* populations. The wide environmental tolerances are characteristic of *R. ficaria*, which would lead me to hypothesize that soils of greater moisture and pH would lead to increased plant performance.

## 2.3 METHODS

### *R. ficaria* Biology

*Ranunculus ficaria* is a perennial buttercup, which is pervasive in river valleys throughout Ohio and threatens vulnerable spring ephemeral communities. A spring ephemeral strategy is unusual for an invasive because they disappear for parts of the year, potentially facilitating the growth of other plants. *Ranunculus ficaria* has an early, yet brief growing season, where it germinates in spring around March and April, and dies off

in the early summer around June and July (Axtell et al., 2010). A large amount of damage can be done during this short window period, as it can spread rapidly, forming thick monocultures as it takes advantage of increased sunlight before the canopy emerges. By getting a head start, it can crowd out native species, which can have a difficult time competing. *Ranunculus ficaria* is not as palatable as its competitors for many herbivores, which contributes to its competitive edge (Axtell et al., 2010).

*Ranunculus ficaria* may be a particularly successful invasive due to its multiple reproductive strategies; besides forming seeds, it also produces tubers and aboveground bulbils. Under intermediate disturbance, *R. ficaria* invests more heavily in the production of seeds (Frederik et al., 2008). Bulbil production is an unusual reproductive strategy, and more studies are required in order to gain a greater understanding of this process. When resources are not readily available for sexual reproduction, asexual bulbils are produced in place of seeds, with the amount of bulbils produced being inversely related to the amount of seeds produced (Arizaga et al., 2000). Tubers tend to have higher germination rates than seeds and often require some sort of mechanism to break dormancy, such as cold stratification (Kertabad et al., 2013). A study conducted in Iran (Kertabad et al., 2013) found tubers were the most important means of reproduction and dispersal. The highest germination rates occurred after cold stratification at two weeks at 4 or 8 degrees Celsius depending on the tuber size (Kertabad et al., 2013).

The degree to which *R. ficaria* produces seeds, bulbils and tubers is variable and may depend on which subspecies is present. Bulbils are thought to have evolved in response to limited pollinator visitations in moist habitats mainly caused by a shady living environment (Deng et al., 2013). Due to tradeoffs in resource allocation, an increase in

clonal investment is linked with decreased allocation to sexual reproduction (Barrett, 2015). Extensive vegetative dispersal and clonal growth can negatively impact the functioning of sexual polymorphisms, which can relate to single mating groups with consequential effects on mating and fertility (Barrett, 2015). Sexual dysfunction and loss of sexual reproductive ability can result from populations where clonal propagation is the dominant form of dispersal that aids mutations which decrease fertility (Barrett, 2015). Seed and bulbil production varies with litter depth; heavy litter makes it difficult for plants to penetrate through and bare ground could cause freezing (Masters and Emery, 2015).

Although *R. ficaria* has been identified as potentially allelopathic, no allelochemical has been identified yet. *R. ficaria* has not been found to have allelopathic or nutrient competition effects when tested against a native riparian grass, *Elymus riparius*. Despite this, significantly greater *E. riparius* biomass and volunteer seedlings were found in plots where *R. ficaria* was removed; implying that *R. ficaria* directly reduces sprout abundance and plays a role in driving ecosystem change (Masters and Emery, 2016). Management implications suggest that the removal of *R. ficaria* populations will help to restore native plant species (Masters and Emery, 2016).

*Ranunculus ficaria* is generally found in soil pH ranges of 4.4 to 6.9 (Taylor and Markham, 1978) with highest occurrences for all five subspecies near a water source (Post et al., 2009). While aboveground-belowground interactions are important to understand, there is a notable lack of information about the effects that soil variables have on plant community traits. Variation in the influence of soil characteristics and plants make generalizing and predicting trends very difficult (Porazinska et al., 2003).

### Detailed transect study

*Location of Study Site:* The seed, bulbil and tuber production rates of were examined along the river valley of the Rocky River Reservation in the Cleveland Metroparks in Cleveland, Ohio (41.40902 N, -81.88399 W). The Rocky River Reservation was chosen to conduct the experiments as it has the highest infestation of *R. ficaria* in Cuyahoga County (Cleveland Metroparks Invasive Plant Atlas, 2016). In 2008 the Rocky River Reservation had over 183 acres of *R. ficaria* where cover was greater than 50% (Mack, 2008). This reservation provides a large array of recreational services from hiking trails and picnic sites to golf courses. It mainly consists of floodplain deciduous forests, wetlands and meadow with a variety of wildlife and bird species year-round. The dominant tree species include willow, sycamore, beech, and maple species. Six sample sites were selected in 2015, with an additional two in 2016, spanning a large portion of the Rocky River in order to explore reproductive performance while ensuring varying site characteristics.

*Site Selection:* The sample sites were selected based upon a location, infestation levels and management history. Sample sites were spaced out enough in order to include differing site characteristics. Sites were located on floodplains and lastly, sites with an undercut bank or not susceptible to flooding events were not considered. The sample sites required a *R. ficaria* population to extend at least up to 25 m away from the river, as is common along this watershed, while attempting to mitigate any anthropogenic disturbances such as trails or paths as much as feasibly possible. Additionally, management history was also taken into consideration, where sample sites preferably had no management in the last two years. This management history criterion excluded many

potential sample sites that were situated in more publicly accessible areas such as easily accessible picnic sites and trails. It is important to note that there were often anthropogenic alterations around 10 to 20 meter quadrats away from the river, in the form of hiking trails or foot paths. At most sites, this was unavoidable due to the extensive network of paths throughout the Rocky River valley.



Figure 2-2: Depiction of my eight sample sites spread out along Rocky River, displayed using Google Maps. There were six sample sites chosen originally in 2015 and two additional sites were added to the study in 2016.

*Experimental Design:* A transect was set up perpendicular to the river at each of six sites in 2015, starting from the vegetation nearest the riverbank, and extending away from the river, to represent a disturbance (flooding) gradient. Twenty cm x 20 cm quadrats were set up along each transect at 0, 5, 10, 15, 20 and 25 meters away from the stream



bank to evaluate the *R. ficaria* aboveground biomass, density and reproductive output (bulbil and tuber production). Within each of these quadrats, ten plants were removed at random coordinates selected using a random number generator. Bulbils and tubers were counted, weighed, and the length of each removed plant was measured. The total number of stems within each quadrat was then quantified to estimate density. In addition to the ten individual plants sampled, ten stems per quadrat were also selected at random from each quadrat for destructive sampling to quantify biomass and bulbil production. The average above ground plant biomass was calculated for the ten randomly removed stems, which were dried for two days at 60 degrees Celsius in a drying oven (Fisher Scientific, Isotemp oven) prior to weighing. All of the plants collected were from the subspecies *bulbifer*.

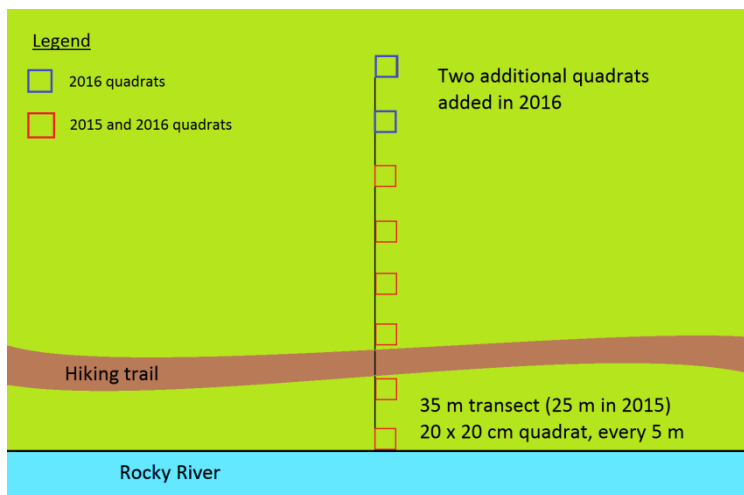


Figure 2-3: An example site set up, using a 35 meter transect running perpendicular away from Rocky River. Every 5 meters along the transect, a 20 x 20 cm quadrat was set up, with the first line of vegetation marking the 0 m starting point. In 2015, the transect was 25 m long, and two additional quadrats were added in 2016 taking the transect up to 35 meters. Hiking trails and swales were often found at distances between 5 and 20 meters away from the river, with varied levels of disturbance.

*2016 Field Season Additions:* In 2015, at some sites it seemed the populations extended beyond 20-25 m, thus I extended the transect length by ten meters in 2016 to gain a greater understanding of the plant dynamics at distances further from the Rocky River. Two additional sample sites were also added. The site selection process followed the same guidelines set in 2015, with an additional goal to include sites south of Site 1.

*Site Characteristics:* The distance away from the river, light availability (PAR or photosynthetically active radiation), soil characteristics (pH, % moisture and nutrient analysis), slope and aspect were recorded at each site. PAR measurements (MQ-306: Line Quantum with 6 Sensors) were aimed to be taken as close to noon as possible on a cloudless day in order to minimize experimental errors. In 2016, two 15 cm soil cores were taken from each quadrat for analysis in the lab. The pH and soil moisture content was measured for each sample. Soil moisture was calculated by comparing the moist soil mass with the dried mass after two days of drying (65°C). Nutrient analyses (P, K, Ca, Mg, CEC, % Ca, % Mg, % K, pH, LTI and total nitrogen) were performed by the STAR laboratory at the Ohio State University. Soil texture (particle size analysis) was also investigated using the hydrometer method.

#### 2016 Large Scale Collection:

A larger-scale sample was collected to examine the accuracy and effectiveness of the predictive plant model described in Chapter 3. This dataset was also analyzed to gain more insight into the impact of site characteristics on *R. ficaria* performance. Sites were selected using a combination of GIS software to generate 30 random points along the Rocky River Valley. RiverTools 4.0.2 (Rivix, LLC, Broomfield, Colorado, USA) was used to extract a shapefile of the Rocky River, which was clipped using ERDAS Imagine

15.0 (Hexagon Geospatial, Huntsville, Alabama, USA) to focus solely on the highest order portions of the river. The random point generator in ArcMap 10.3.1 (Environmental Systems Research Institute, Redlands, California, USA) was used to identify 30 random sample points along the river. All site collections were done on the west bank of Rocky River except for two sample sites that were inaccessible due to sheer cliff faces and private property.

At each sample site, a 30 meter transect was set up as close to the randomly generated point as feasibly possible, perpendicular to the river (avoiding large fallen trees, paths or any other major impedance on transect.) Sampling was conducted at the 0, 15 and 30 meter marks along the 30 meter transect, representing close, intermediate and far distances from the river. The percent cover and dried above ground biomass were measured at each distance of the three distances away from the river. Site characteristics measured include canopy cover, PAR, slope angle and aspect. Pictures were taken of quadrats along each transect. At each sampling location along the transect, a 2 x 2 meter quadrat was set up, using the flag as the bottom right corner of this quadrat for a reference point, with the observer's back towards the river. Within these three 2 x 2 meter quadrats, percent cover was estimated using Daubenmire (1959) cover classes: 0-5 %; 5-25 %; 25-50 %; 50-75 %; 75-95 %; 95-100 %. *Ranunculus ficaria* biomass was collected from 50 x 50 cm quadrats at each sampling location. Biomass samples were then dried in the oven for two days at 60 degrees Celsius and weighed.

*Analysis:* All analyses were conducted using R (R Core Team, 2013). Regression models and linear mixed effect models were used to examine bulbil and tuber production (counts, mass, length), plant biomass, density and percent cover in relation to distance

away from the river. Analyses also explored additional site characteristics such as soil pH, soil moisture, soil nutrients, aspect, slope, PAR and canopy cover.

The data sets contained a large amount of null values so additional computations were done in order to eliminate the possible influence of zero inflation (Martin et al., 2005). To standardize the values for better comparisons of bulbils and tubers, additional variables were calculated such as bulbil mass and length per bulbil, bulbil count per bulbil producing plant, tuber mass and length per tuber, as well as tuber count per tuber producing plant. For the large-scale collection data set, zero inflation was handled by omitting zero values during model fitting (Martin et al., 2005). The critical value used to determine significance was 0.05.

## 2.4 RESULTS

A high amount of variability in plant performance was observed between sample sites during both years of field observations. The mean dry plant biomass for a quadrat was 0.5661 g (max=2.80, median=0.4863). All maxima in 2015 were recorded from site 1, which was the most productive site. In 2016, two additional sites were added, and were similar in productivity to Site 1. Bulbil production ranged from 0 to as high as 18 per plant (mean = 2.851) and tuber production ranged from 1 to 11 tubers per plant (mean = 2.417). Densities of *R. ficaria* were found to be as high as 11,425 plants/m<sup>2</sup> in some areas with an overall mean of 2,772 plants/m<sup>2</sup>. Interestingly, there was no seed production observed in any of my sample quadrats.

The *R. ficaria* distribution was generally sparse near the river (0 m quadrats) and then a dense monoculture was observed up to the 20 m quadrat. Beyond the 20 m quadrat,

there appeared to be higher abundances of other species present. Some sites had a vegetative mat of *R. ficaria* that extended beyond the farthest quadrat (25 m), primarily in locations consisting of a well-established, successful population of *R. ficaria*. Sites with a gentle gradient were most likely to have extended populations beyond the length of the established transect. Sites that would rapidly increase in gradient beyond the transect had a decline in all vegetation, not just *R. ficaria*, and steep slopes are often common features throughout river valleys due to erosion over time. In similar *R. ficaria* populations (Sites 3 and 4), those situated on north facing slopes experienced withering and decay earlier than populations on south facing slopes. I observed much higher densities and bulbil counts during the 2016 field season than in 2015. Plant biomass recorded in 2016 was similar to collections in 2015.

Soil pH levels appear to influence plant performance, *R. ficaria* favoring higher pH soils. Plant biomass had a significantly positive relationship with soil pH, where higher pH soils saw increased plant biomass (Regression,  $F_{1,62} = 7.799$ ,  $P = 0.007$ , Figure 2-4a). Bulbil counts per stem also had a positive significant relationship with soil pH (Regression,  $F_{1,30} = 4.602$ ,  $P = 0.040$ , Figure 2-4b).

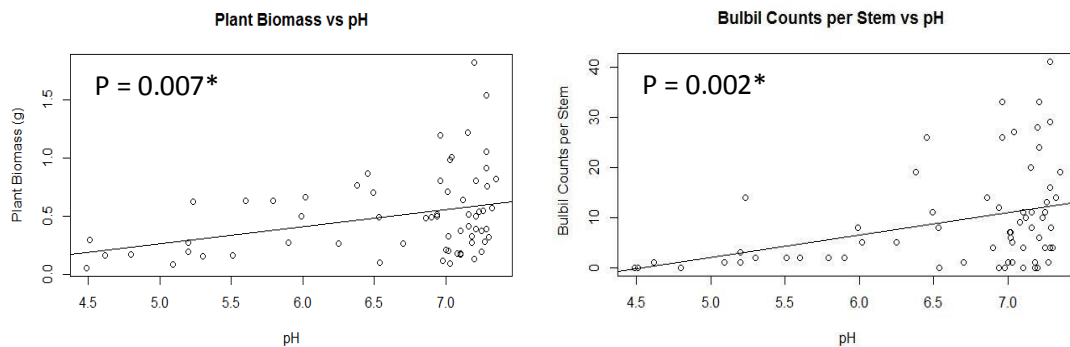


Figure 2-4 a and b: Plant biomass and bulbil counts as a function of soil pH for the soil analysis experiment using soil data collected in 2016 (a). Bulbil counts per stem versus

pH for 2016 (b). Plant biomass had a positive linear relationship with pH, indicating greater plant biomass at sites with greater pH values (Regression,  $F_{1,62} = 7.799$ ,  $P = 0.007$ ). There was a significant positive relationship found between bulbil counts per stem and pH, where higher counts were found in areas of higher soil pH (Regression,  $F_{1,30} = 4.602$ ,  $P = 0.002$ ).

LTI (lime test index), which is an indication of the buffer quality of soil, was significantly associated positively with *R. ficaria* plant biomass, bulbil counts per stem and tuber counts (Figure 2-5). Higher values of LTI mean that less lime is required to bring the soil up to a neutral level.

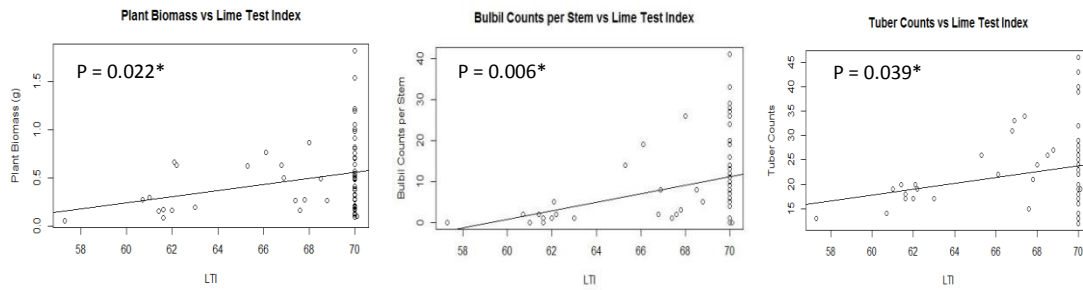


Figure 2-5 a, b and c: LTI levels in the soil versus plant biomass (a), bulbil counts per stem (b) and tuber counts (c) respectively. There were significant positive relationships between LTI levels with plant biomass, bulbil counts per stem and tuber counts (Regression,  $F_{1,62} = 5.548$ ,  $P = 0.022$ ; Regression,  $F_{1,62} = 8.022$ ,  $P = 0.006$ ; Regression,  $F_{1,62} = 4.47$ ,  $P = 0.039$ , respectively).

*Ranunculus ficaria* appeared to thrive in areas of high nutrient availability, which can be noticed through both increased biomass and reproductive outputs. Phosphorus and calcium were both identified through the soil analyses in 2016 as key nutrients, which resulted in greater plant biomass and reproductive output when found in greater abundances in the soil (Figure 2-6 and 2-7). These nutrients are important for the healthy growth of plants and may play an integral role in determining successful *R. ficaria* populations, along with additional secondary soil nutrients such as potassium and magnesium.

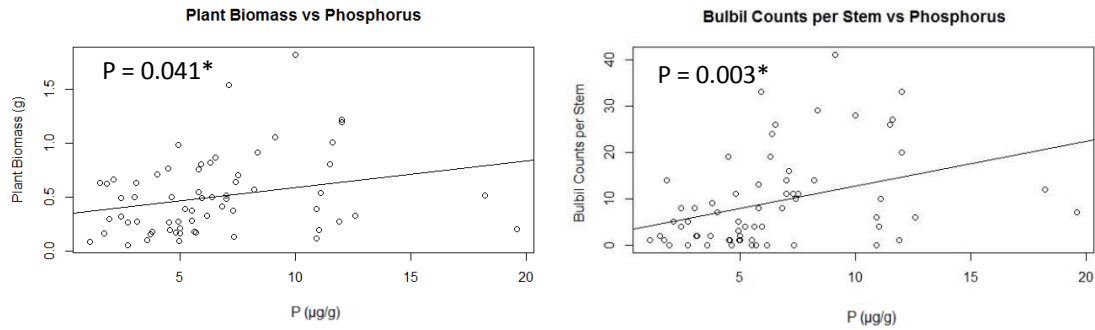


Figure 2-6 a and b: Phosphorus levels in the soil versus plant biomass (a) and bulbil counts per stem (b) respectively. There were significantly positive relationships between phosphorus with plant biomass and bulbil counts per stem respectively (Regression,  $F_{1,62} = 4.364$ ,  $P = 0.041$ ; Regression,  $F_{1,62} = 9.649$ ,  $P = 0.003$ ).

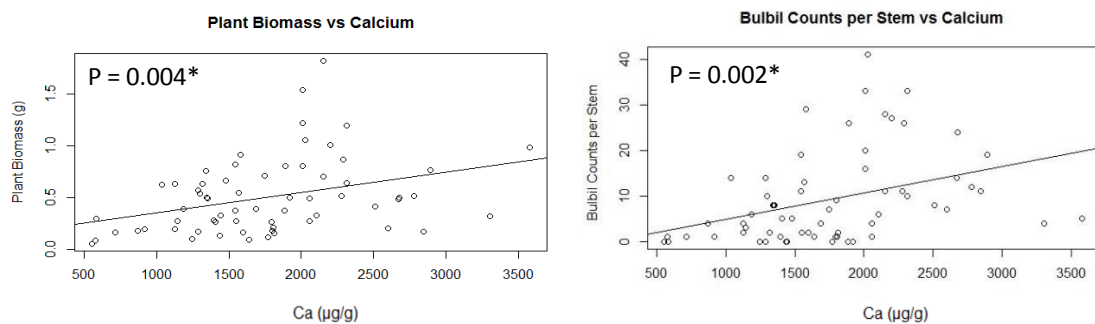


Figure 2-7 a and b: Calcium levels in the soil versus plant biomass (a) and bulbil counts per stem (b). Regression for figures 2-7 a and b resulted in significantly positive relationships between calcium levels with plant biomass and bulbil counts per stem respectively (Regression,  $F_{1,62} = 8.678$ ,  $P = 0.004$ ; Regression,  $F_{1,62} = 9.883$ ,  $P = 0.002$ ).

Soil texture appears to influence the availability of calcium in the soil (Figure 2-8), which is a key nutrient for *R. ficaria* growth and proliferation (Figure 2-7). Calcium levels were found to decrease with sandier soils, while being found to increase in abundance in soils with higher percent clay and silt (Figure 2-8). Bulbil counts per individual plants were significantly lower in sandier soils (Figure 2-9). There was a significantly positive relationship between bulbil counts per individual plants, tuber counts and tuber mass with percent silt (Figure 2-9). These findings reiterate the importance of calcium levels in the soil for *R. ficaria* performance. Plant biomass and

bulbil counts per stem appeared to increase with higher percent clay and silt, yet the relationships were not significant. Generally, soils closer to the river had higher percent sand, while those further away had higher percent clay and silt. These observations matched what was seen in the field, yet no significant trends were found between soil texture and pH or distance away from the river.

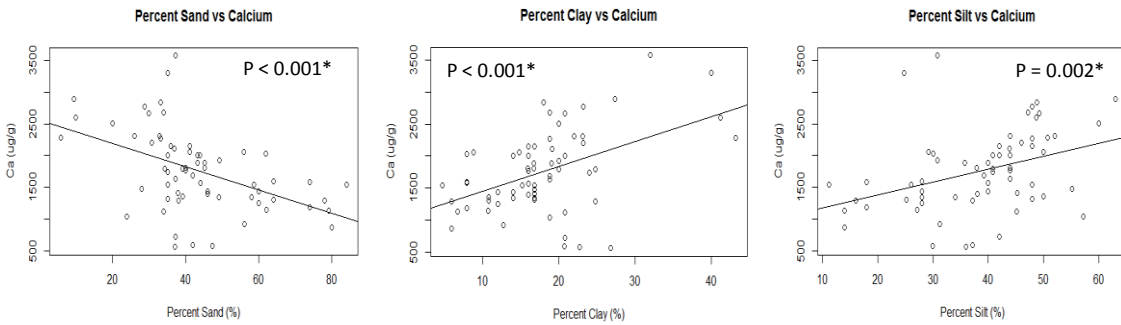
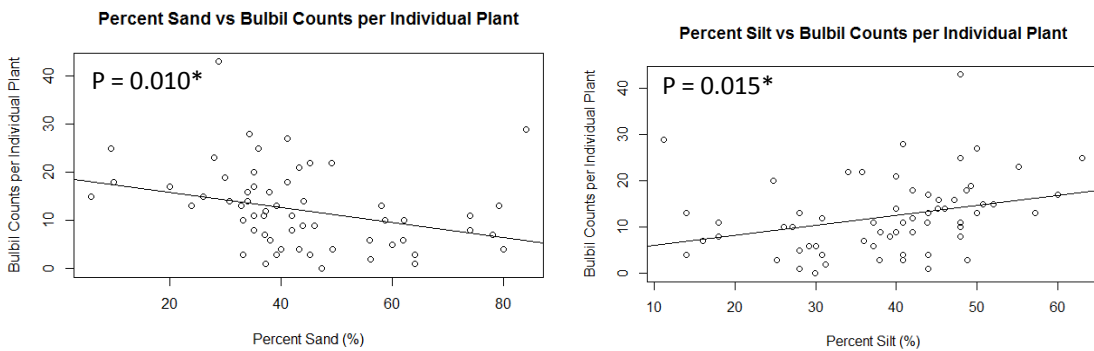


Figure 2-8 a, b and c: Percent sand (a), percent clay (b) and percent silt (c) versus calcium levels in the soil for 2016. There was a negatively significant relationship between percent sand and calcium levels (Regression,  $F_{1,62} = 18.290$ ,  $P < 0.001$ ). There were significant positive relationships between both percent clay and percent silt with calcium levels in the soil (Regression,  $F_{1,62} = 17.950$ ,  $P < 0.001$ ; Regression,  $F_{1,62} = 9.744$ ,  $P = 0.002$ ).





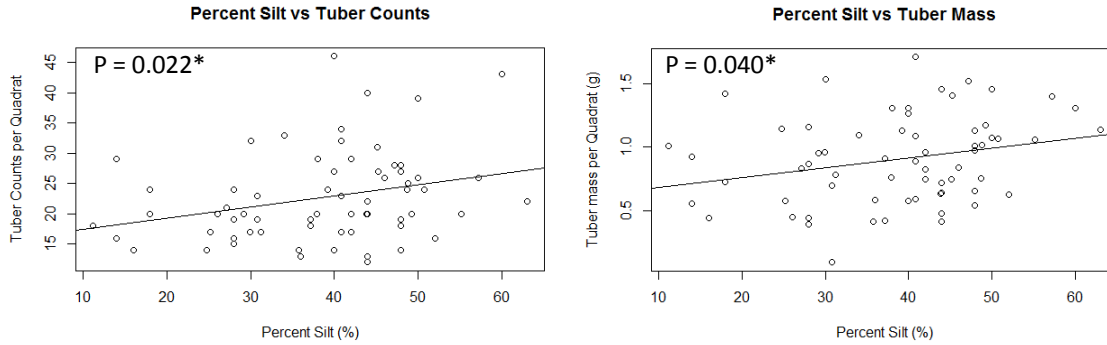


Figure 2-9 a, b, c and d: (a) Percent sand versus bulbil counts per individual plant. Percent sand displayed a negatively significant relationship with bulbil counts (Regression,  $F_{1,62} = 6.989$ ,  $P = 0.010$ ). Percent silt versus (b) bulbil counts per individual plant, (c) tuber counts per quadrat and (d) tuber mass per quadrat had positively significant relationships (Regression,  $F_{1,62} = 6.242$ ,  $P = 0.015$ ; Regression,  $F_{1,62} = 5.514$ ,  $P = 0.022$ ; Regression,  $F_{1,62} = 4.412$ ,  $P = 0.040$ , respectively).

Unexpectedly, the distance away from the river did not seem to influence the plant performance or reproductive outputs of *R. ficaria*. *R. ficaria* density was observed to have a mean of 2,772 plants/m<sup>2</sup> (max=11,425, median=2412). This maximum was an outlier, with a large number of tiny *R. ficaria* plants.

Average bulbil production ranged from 0 to 6.1 per stem (mean=1.306, median=0.950) and average bulbil production per individual plant ranged from 0 to as high as 4.5 (mean=0.736, median=0.800). The bulbil counts for whole individual plants were carried out after the counts per stem in both years. It appears that bulbil germination rates by the end of the year are extremely high, indicating little innate dormancy in the field. Bulbil counts per stem did not vary significantly with distance from Rocky River (Regression,  $F_{5,66} = 0.521$ ,  $P = 0.759$ , Figure 2-10). The variability of reproductive output tended to be greatest at intermediate distances away from the river (Figure 2-10). The highest values were often observed at the 15 m quadrat. 31.11% of the plants sampled over the two years produced bulbils, which was much lower than the 99.03% that

produced tubers. The average number of bulbils found on a bulbil producing plant was 2.72. The average bulbil length was 3.8838 mm, with an average mass of 0.0125 g.

The average number of tubers found on a tuber producing plant was 2.46. The average tuber length was 7.9460 mm, with an average mass of 0.0408 g. While the median individual tuber masses appear to be greater, with lower variability at intermediate distances from Rocky River, there was no significant relationship found between the two variables (Regression,  $F_{5, 66} = 0.825$ ,  $P = 0.537$ , Figure 2-10). There was a significant trend between tuber production and LTI (Regression,  $F_{1, 62} = 4.473$ ,  $P = 0.0385$ , Figure not included). Tuber production ranged from 1 to as high as 11 tubers per plant (mean = 2.417, median=2.150).

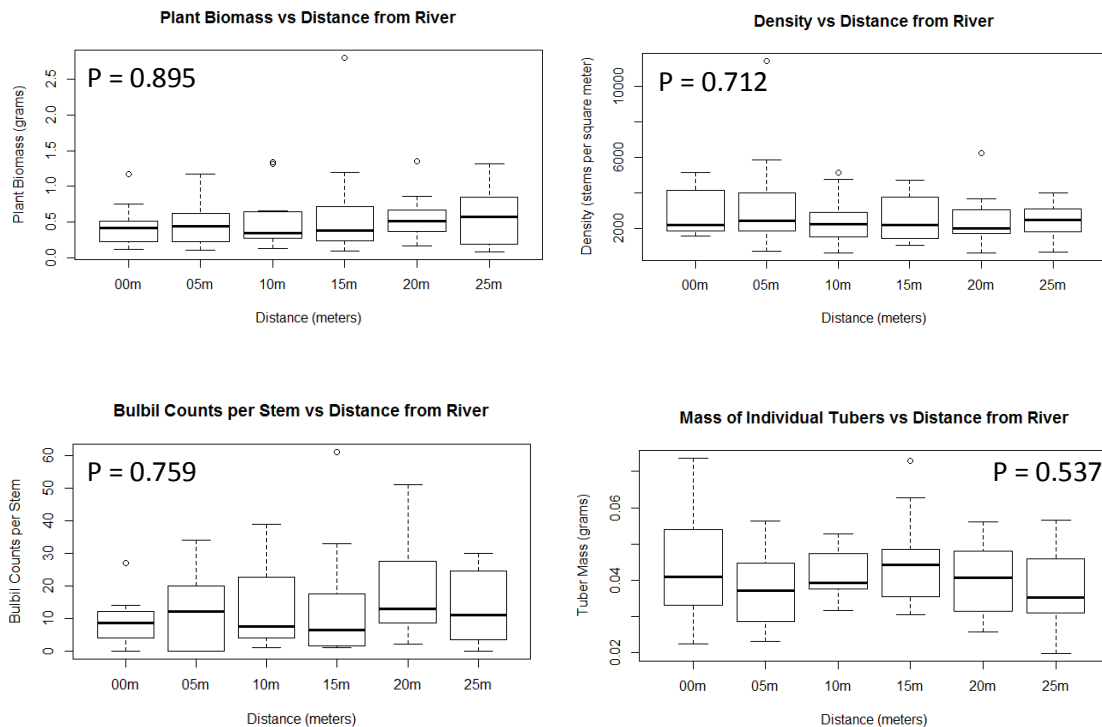


Figure 2-10 a, b, c and d: (a) Boxplots of above ground biomass versus distance away from the river for 2015 and 2016 combined. There is no significant trend in plant biomass with respect to distances away from the river (ANOVA,  $F_{5, 66} = 0.327$ ,  $P = 0.895$ ). (b)

Boxplots of density (stem count per square meter) versus distance away from the river for both 2015 and 2016 combined. Groups were not significantly different from each other indicating no significant trend in plant biomass with respect to distances away from the river (ANOVA,  $F_{5, 66} = 0.584$ ,  $P = 0.712$ ). (c) Boxplots of bulbil counts per stem versus distance away from the river for both 2015 and 2016 combined. There was no significant trend in plant biomass with respect to distances away from the river (ANOVA,  $F_{5, 66} = 0.521$ ,  $P = 0.759$ ). (d) Tuber mass along a disturbance gradient away from the river. Tuber mass showed no significant relationship with plant biomass (ANOVA,  $F_{5, 66} = 0.825$ ,  $P = 0.537$ ).

*Large scale observational study.* Distance from the river did not seem to influence plant performance in this large scale study either. There was no significant trend between plant biomass with distance away from Rocky River (Figure 2-11). The maximum value was found at the 15 meter quadrat, similar to the main study.

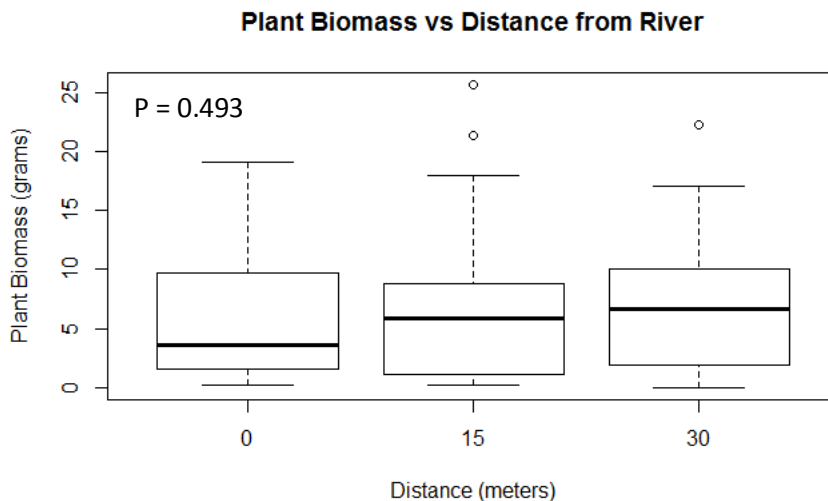


Figure 2-11: Boxplots of above ground biomass versus distance away from the river for the larger scale collection in 2016. The groups were not significantly different from each other, indicating no significant trend in plant biomass with respect to distances away from the river (ANOVA,  $F_{1, 46} = 0.478$ ,  $P = 0.493$ ). This figure uses data points from quadrats where *R. ficaria* was present.

According to the larger scale observational study in 2016, slope angle appeared to impact the plant performance of *R. ficaria*. Slope angle had a significant linear

relationship with plant biomass (Figure 2-12) indicating that the slope gradient of sites may play an important role as a driving force behind the plant performance and degree of infestation. Plant biomass maxima were found at locations of low slope gradients of below ten degrees. Slope face direction was also analyzed through radial plotting, yet aspect showed no significant trend in relation to plant biomass ( $p = 0.216$ ).

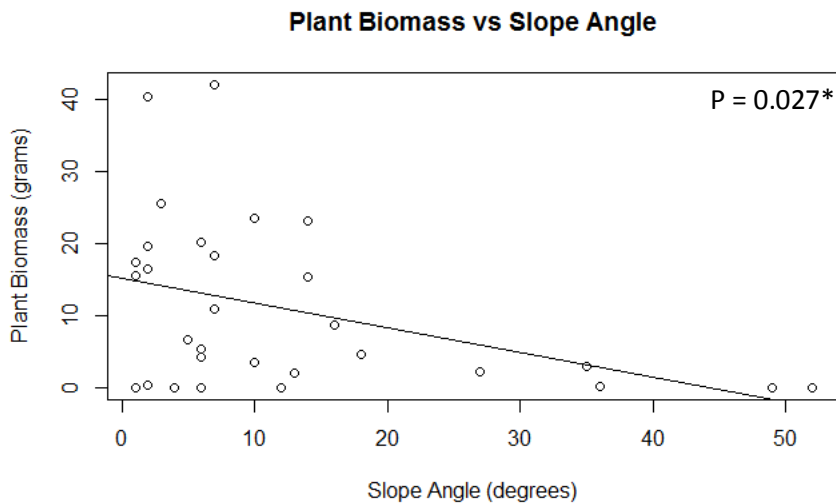


Figure 2-12: Above ground plant biomass plotted against slope angle in the larger scale observation in 2016. Slope angle had a significant linear relationship with plant biomass (Regression,  $F_{1,28} = 5.401$ ,  $P = 0.027$ ). *Ranunculus ficaria* appears to thrive in areas of lower gradient.

Canopy cover appears to play an important role in plant performance according to the larger scale study. Percent cover was significantly related to percent canopy cover (Figure 2-13). This is interesting as there was greater percent cover of *R. ficaria* in shadier areas. Plant biomass showed a marginally significant relationship with percent canopy cover (Figure not included).

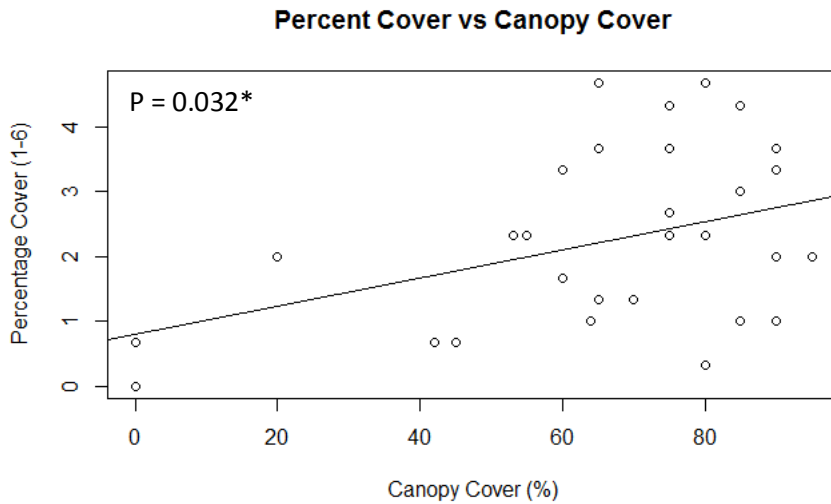


Figure 2-13: Percent cover was significantly positively related to canopy cover as measured during the large scale collection in 2016 (Regression,  $F_{1,28} = 5.401$ ,  $P = 0.032$ ). *Ranunculus ficaria* appears to thrive in more shady areas.

PAR measurements were taken throughout the main study as well as the larger scale sampling. No significant relationships were found overall with the combined data set, although using just the data from the 2015 field season, PAR was found to be statistically significant ( $p = 0.0258$ ) using mixed effects models, factoring site as a random variable.

## 2.5 DISCUSSION

Soil characteristics are important driving forces behind *R. ficaria* success, with significant positive relationships between plant characteristics and pH, LTI, phosphorous, calcium, magnesium, percent calcium and percent silt. These characteristics can be used to identify high risk populations for immediate management.

While some sites did have better plant performance at intermediate distances, overall, there were no significant relationships between plant performance and distance, failing to support the second hypothesis. One possible reason for why plant performance was not

higher at intermediate distances is that at several sites, flooding from the river was not the only major disturbance. There were several trails and swales that generally occurred around the 10 – 20m quadrats, and these tended to have poor plant performance.

Anthropogenic disturbances were avoided as much as possible, yet Rocky River is an extremely popular and well frequented park by the public, making certain disturbances unavoidable. This resulted in many intermediate distance plots with high disturbance patterns.

The most productive sites, in terms of total plant biomass and reproductive output (bulbil counts per stem), generally had higher pH values with lower variability, as well as higher soil moisture. Conversely, the least productive sites displayed the lowest mean soil moisture and pH, while having greater variability observed for both variables. The soil pH became increasingly acidic with distance away from the river and those sites that experienced a more gradual decline had higher total plant biomass. Habitat requirements for *R. ficaria* require a pH range of 4.4 to 6.9 (Axtell et al., 2010). The *R. ficaria* populations during my two year observational study had a pH range of 4.49 to 7.35, and the healthier populations were found on soils with the highest pH values. This elevated pH range may be attributed to the parent rock material in the Cleveland area, which can result in soil pH above 7.0, with the higher pH areas generally at greater risk of invasion (Tsui et al., 2004). Increasing soil acidity allows for increased availability of phosphorous uptake, yet alkaline elements such as potassium, magnesium, calcium and sodium are lost (Petra and Proctor, 2001). This may explain why *R. ficaria* prefers more basic soil, as calcium had a positively significant relationship with both plant biomass and bulbil counts (Figure 2-7). Soil texture was also linked to calcium levels in the soil, with

concentrations significantly higher in high percent silt and clay soils (Figure 2-8). Plant performance variables such as bulbil counts, tuber counts and tuber mass were found to be significantly higher on higher percent silt soils, which may be due to elevated calcium levels in these soils. This is not surprising as calcium has been linked to increased plant performance and number of invasive species (Harrison, 1999; Howard et al., 2004). These trends imply an important role that soil characteristics may play in determining the plant performance of *R. ficaria*. *Microstegium vimineum* is another example of a shade-tolerant, invasive plant species that performs better at higher soil pH levels (Anderson et al., 2013). Human activity can alter soil pH, which can in turn facilitate invasive species spread over native species (Tilman and Lehman, 2001).

Swales were another common site feature at intermediate distances along each transect, and were often too saturated and muddy for any vegetation to be successful. Sites varied greatly and were individually examined in addition to the primary analyses (Figures not shown). Self-thinning (Westoby, 1984) was observed in the field and was likely occurring, where larger, more networked plants were at intermediate distances from the river, thus requiring greater resources. Close to the river, plants appeared to be single or double stemmed. As plants grow larger in height, with additional lateral growth, they require more space and resources, thus thinning out intermediate areas to some degree (Westoby, 1984). There appeared to be greater plant biomass recorded where plants occurred at lower densities, although the trend is not significant (Figure not shown).

Canopy cover for the larger scale study had a positive significant relationship with *R. ficaria* percent cover, and plant biomass appeared greater with increasing canopy cover,

although this relationship was only marginally significant (Figure 2-13). These results may be due to lower canopy cover close to the river, where *R. ficaria* populations generally were less dense than further along the transect. Canopy cover was greater further away from the river where there appeared to be slightly higher biomass measurements. This may explain the positive relationship between plant biomass and canopy cover. Light availability is a factor that can often play an important role in plant growth and proliferation (Meekins and McCarthy, 2000). *Ranunculus ficaria* is most often found in shaded sites and less commonly found in full sun on open ground, unless soil moisture levels are sufficient (Axtell et al., 2010). Too much direct sunlight may also lead to withering in populations, and thus slightly shaded areas often seemed to relate to more vigorous populations in the field. *Ranunculus ficaria* is a spring ephemeral that starts growing well before leaf-out, when shaded areas are less prevalent. When the canopy cover gets extremely thick due to tall dense trees, this can result in poor conditions for ground vegetation of all types. If there is minimal canopy cover, the soil may dry out, resulting in unfavorable growing conditions for the floodplain invasive.

Slope has a significantly negative relationship with percent cover and plant biomass (Figure 2-12). It is clear in the field that, although *R. ficaria* can be found on very steep slopes, the plant population is increasingly sparse and patchy with increasing gradients. Steep slopes generally have dry, shallow soils and *R. ficaria* prefers moist, fertile floodplain (Axtell et al., 2010). Invasive plants occur most frequently on flatter slopes, with other herbaceous plant species (Underwood et al., 20014).

Water level fluctuations can influence effects on plant performance, which allow some plant species to thrive in areas of high frequency flooding while restricting others to



higher elevations (Leyer, 2005; Van Eck et al., 2004). Variations in weather patterns from year to year can play a significant role in observational studies of this nature. Ohio's weather conditions can change dramatically from year to year, and the past two years show large temperature and precipitation differences from 2015 to 2016. The 2015 winter was significantly more severe, with temperatures reaching much colder levels with greater amounts and sustained snow and ice cover. The cold winter rebounded sharply into a warmer spring to summer transition in comparison to 2016. There were just over three months from January 2015 with no discharge data recorded for the Rocky River, indicating ice cover, which later caused sharp and erratic flash floods after ice melt. The 2016 discharge is a lot more consistent and deviates less from the median daily statistic in comparison to the erratic 2015 field season. These irregular and more intense flooding events could help to spread the *R. ficaria* propagates to a greater degree as the floodplain experiences greater disturbances. There was a high level of variability observed during the two year observational study. I observed similar plant biomass with much higher densities and bulbil counts in the 2016 field season than in 2015. Both plant density and bulbil counts were almost doubled, and this could be attributed to more favorable temperatures for plant growth during 2016. *R. ficaria* prefers cooler growing conditions and starts to senesce with tubers delaying their growth when temperatures consistently hit or exceed 20 degrees Celsius (Kertabad et al., 2013). This implies that weather conditions may have played a large role in the significantly increased reproductive output in 2016. In general, spring ephemerals prefer cooler growing conditions, which result in longer leaf longevity (Badri et al., 2007; Yoshie, 2008).

During the two year observational study in Rocky River Reservation, *R. ficaria* barely produced any seeds. This suggests that *R. ficaria* populations in Rocky River do not rely heavily on sexual reproduction. Resource allocation in this invasive favors asexual reproduction, through high production of tubers and above-ground bulbils. Whether this is due to self-incompatible populations of clones or other selecting forces are unknown and would be an interesting research direction for further investigation. Vegetative diaspores have been hypothesized to be physiologically advantageous during germination (Jung et al., 2008). This may be a more effective method for dispersal as germination rates have resulted in higher percentages for bulbils than seeds, with 81% germination for bulbils in comparison to 71% germination for polyploid seeds and 18% for diploid seeds (Marsden-Jones, 1993). One drawback of investment into tubers and bulbils is the high resource cost needed to produce the large structures, yet field observations show that the results are likely worth the cost with successful infestations of *R. ficaria* relying asexual reproduction.

Management will be most effective in combating population spread by targeting sites where high reproductive output is likely. Soil characteristics appear to play a large role in driving population performance and should be considered to help prioritize the allocation of resources for management. Besides reproductive potential, it is also important to consider where spread is most likely to occur. Areas close to the river are extremely important with regards to population spread and may also require management. Overall, the highest priority sites are in a floodplain with high pH, soil moisture, potassium and calcium content in the soil, with a low slope gradient and moderate to high canopy cover.

Further research is necessary to gain a greater understanding of this troublesome invasive, with the bulbil dormancy being one of the major concerns. Additional variables that could prove to be fruitful for further study include the impacts of leaf litter depth, mycorrhizal associations, grazing, pollinator limitation, seed predation, competition and community interactions with other species. This study was able to expand on the current limited understanding of *R. ficaria*, which can lead to more efficient management and control of its spread.

## CHAPTER III

### GIS MODEL OF LESSER CELANDINE (*RANUNCULUS FICARIA* L.) PLANT PERFORMANCE

#### 3.1 ABSTRACT

Effective invasive plant management requires a thorough understanding of where invasives are found and where they are likely to grow vigorously and spread. *Ranunculus ficaria* is an invasive buttercup from Europe, which has infested a significant proportion of North America, especially the northeastern parts of the United States. This herbaceous perennial can cause negative environmental impacts and is thus a high management priority. It is important to better understand which areas are most suitable for *R. ficaria* growth to prioritize management efforts. Not all populations of an invasive plant are capable of rapid growth, and it is important to allocate limited resources toward controlling critical populations. The aim of this project was to create an accurate and reliable classification that could be used to predict the performance of *R. ficaria* in the field. I expected to achieve higher accuracies predicting high performance plots. I also

expected more accurate predictions for plots closer to the river, where sites characteristics are more uniform, with lower species richness. Using Definiens eCognition, an object-based image analysis approach was used to create a habitat suitability model of *R. ficaria* population density. A multiresolution segmentation was carried out on high resolution color-infrared Unmanned Aerial Systems (UAS) imagery, a mixture of spectral and topographical metrics were used to classify the Rocky River. Variables included in the model were soil type, slope angle and aspect. In order to evaluate the accuracy and effectiveness of the generated classification, a field survey was conducted. Thirty randomly selected sites were sampled throughout the length of the Rocky River, where biomass, percent cover and site characteristics were taken along 30 meter transects. The field survey was used to categorize sites into high, medium and low plant performance in order to ground-truth the GIS model. The plant performance model had an accuracy of 68%, which is consistent with work on similar understory species (Hantson et al., 2012; Rego and Koch, 2003). There was no significant relationship between prediction accuracy and distance away from the river. There were significant differences between the expected and observed accuracy of plots categorized by plant performance. However, the trend opposed the hypothesis, with low performance plots achieving greater accuracy. Therefore, I cannot support either hypothesis. While not sufficiently accurate enough for application, the modeling approach can be improved upon through incorporating higher resolution imagery and including additional variables into the classification. This method was able to demonstrate a time effective way to analyze invasive plant populations, which can be helpful in the management of invasive plants.

### 3.2 INTRODUCTION

Invasive species can cause significant environmental, economic and human health impacts with severe consequences documented on a global scale (Pimentel et al., 2005). Invasive plants threaten native communities and can alter ecosystem function, degrade the environment and reduce biodiversity (Jose et al., 2014). Some plant species have been known to cause physical harm to humans at varying degrees (Jose et al., 2014).

It is important to note that not all alien species cause harm, and potential risks are species and community dependent. Invasive plant species are not always invasive in all ecosystems, and there is always variability in invasiveness even across the established habitats (Rew et al., 2005). In more isolated established invasions, invasive plant species will often go locally extinct on their own and not cause a problem (Smith et al., 2006). Management goals should focus on prioritizing control and managing populations to a degree that does not adversely impact the integrity of the ecosystem (Jose et al., 2014). An example of this management technique is the case of the gypsy moth (*Lymantria dispar*), where efforts have been concentrated on slowing down the spread. Small populations are often ignored as they have the potential of disappearing without the intervention and expenditure of valuable resources (Sharov et al., 2002).

Land managers are tasked with the difficulty of trying to prevent or limit damage from harmful invasions with a finite amount of resources and lack of knowledge of the full potential risk a species poses (Jose et al., 2014; Maxwell et al., 2009). This makes management optimization a fundamentally crucial aspect of land managers' responsibilities. For efficient management, it is essential to understand where to manage, based upon the most reliable data sources possible, and to prioritize limited resources and

effort to target strategically key invasive populations. In recent decades, technological advancements have led to an increased attention, usage and availability of powerful software in the field of ecology. Satellite imagery for example has been globally accessible since 1972 (Joshi et al., 2004). Geographical information systems (GIS) and remote sensing (RS) programs allow for extensive modeling capabilities through the use of imagery, made available through both Unmanned Aerial Systems (UAS) and manned aerial photography. Predictive models can be powerful, time and cost effective methods for land managers to paint a clearer picture of what is going on in a particular area at different scales. There are many well-documented benefits to using GIS and RS tools, such as the ability to study inaccessible ecosystems, complex terrain types, multispectral and multitemporal data in a cost effective manner (Joshi et al., 2004). GIS and RS based approaches have been exceptionally useful in the field of invasion for natural resource managers, ecologists and conservationists, especially when used to predict population dynamics and potential areas at risk of invasion (Joshi et al., 2004). The most commonly generated maps have investigated actual invader distribution, potential distribution and areas at risk of invasion (Joshi et al., 2004). Technology has also been used to select high priority management sites, which can reduce the amount of herbicide needed in agriculture. These tools provide land managers with many cost-effective methods of modeling, which can aid them in their difficult task of prioritizing the allocation of limited resources for managing populations of concern. Site specific weed management can have huge economic and ecological benefits, through reducing the usage of pesticides by 54% on average (Timmermann et al., 2003). The benefits are strongly crop and year

dependent, yet using maize as an example, saw a saving of 42 euro/ha (Timmermann et al., 2003).

There are many factors that can influence the accuracy of GIS based projects. Remote sensing methods have been used with variable success in ecology, with many studies producing accurate and expedient results (Rew et al., 2005; Ghioca-Robrecht et al., 2008; Michez et al., 2016; Van Lier et al., 2009). The most frequently used remote sensed data continues to be high resolution aerial photographs (Van Lier et al., 2009). Higher resolution imagery can be expensive and time consuming, yet generally leads to higher accuracy values (Franklin et al., 1994; Van Lier et al., 2009). Studies can vary substantially in success depending on the species of interest, imagery sources and resolutions, and differences in methodologies and expertise (Joshi et al., 2004). A methodology that has demonstrated success involves distinguishing different classes of cover (low, moderate and heavy), where Franklin et al. (1994) were able to use high spatial resolution imagery to achieve high accuracies ranging from 87-99%. In addition to the superior accuracy of using high resolution images, object-based image analysis (OBIA) has demonstrated greater accuracy in comparison to traditional pixel-based classification methodologies (Batz and Schäpe, 2000; Rego and Koch, 2003). Polygon-based multi-resolution segmentation is effective at delineating land cover types through its bottom-up approach (Hess et al., 2003). Inclusion of LIDAR data has been found to increase the accuracy from 39% to 50%, and OBIA further raised the accuracy from 50% to 60%, resulting in substantial improvements in accuracy (Hantson et al., 2012). This shows how RS and OBIA inclusion can substantially increase accuracies, resulting in improved classifications.



The type of species modeled plays a critical role in project success, and RS has predominantly been applied to canopy dominant species (Joshi et al., 2004). The large majorities of invaders (flora and fauna) do not dominate the canopy and due to their increased difficulty in mapping have received much less attention in the application of RS techniques (Joshi et al., 2004). While the mapping of understory vegetation using direct RS techniques is almost impossible, the development of models and risk maps using a combination of GIS, RS and expert knowledge does offer the potential of detecting understory invasions (Joshi et al., 2004). More recently, efforts have been made to overcome the challenge of mapping understory species, yet due to overstory canopy interference, mapping attempts and detailed distribution information has remained limited (Singh et al., 2015; Tuanmu et al., 2010). Studies have found that the inclusion of tracked phenological characteristics from a time series of imagery can be used to improve the mapping of understory species in various geographic areas, especially when elevation information is incorporated (Tuanmu et al., 2010). Another approach using image differencing, where one image is subtracted from another, has been used to successfully improve Amur honeysuckle cover predictions (Wilfong et al., 2009). For this approach to be used it is necessary for the invasive species to have a distinct leaf phenology from native species. Wilfong et al. (2009) found that the Normalized Difference Vegetation Index (NDVI) was the best performing predictor variable. Overall, understory species mapping is still challenging and rarely done, yet depending on the phenology of the species there are opportunities for improving classifications and predictions.

While the ability to remotely survey difficult to access areas is a major benefit to using GIS and RS models, fieldwork is often a necessary component to the project by

informing the modeling process and assessing the accuracy of the model predictions. Basic reproductive information is also necessary to inform demographic models (Crouse et al., 1987; Jongejans et al., 2008), and separate surveys or collection are necessary to assess the accuracy of actual occurrence and plant performance maps (Van Lier et al., 2009; Michez et al., 2016). This is a vital step in the process as it will indicate how useful the model will be in practical application.

In Ohio, there are currently 19 species listed as noxious weeds according to the USDA Plants Database. One particular species of concern, especially in Cuyahoga County, is a perennial invasive known as lesser celandine (*Ranunculus ficaria* L.), which has been spreading rapidly throughout North America for decades. *Ranunculus ficaria* was studied in this research project due to a lack of understanding coupled with land manager interest in this invasive species, with the aim of identifying critical populations.

*Ranunculus ficaria* is a problematic spring ephemeral from the buttercup family. This harmful invasive was brought over to North America from its native ranges of Europe and parts of Asia for ornamental use (Axtell et al., 2010). It is an attractive horticultural plant due to its beautiful yellow flowers and early spring germination. Over the past few decades this harmful invasive seems to have transitioned out of the lag phase of population growth and has rapidly gained the attention of researchers and land managers (Post et al., 2010). It has multiple forms of reproductive output including seeds, tubers and above ground bulbils. This arsenal of reproductive strategies, coupled with its early growing season whereby it takes advantage of light availability before canopies regrow, enable this invasive to rapidly take over floodplain lowlands, often resulting in large monocultures of dense *R. ficaria* mats covering forest floors. This method of proliferation

allows *R. ficaria* to crowd out native species and can result in decreased biodiversity (Swearingen, 2005). *Ranunculus ficaria* thrives in moist lowland areas and is believed to spread via flooding events, making native floodplain populations especially vulnerable to invasion (Post et al., 2010). The Rocky River Reservation in Lakewood, Ohio has a severe infestation of *R. ficaria*, making the area a suitable site for study. In order to effectively manage and control this infestation, it is critical to have a greater understanding of *R. ficaria* population dynamics in different environmental conditions. As a more recent invader, the literature is sparse in terms of understanding the mechanisms for *R. ficaria* invasion success. The greater the understanding we have of *R. ficaria*'s population dynamics, the more effective the management will be of using the limited available resources and time to control its spread and environmental damage.

To investigate which site characteristics play the greatest role in explaining the Rocky River *R. ficaria* infestations, I used a GIS-based model to predict *R. ficaria* plant performance. Using Definiens eCognition, I carried out an object-based image analysis (OBIA) (Baatz and Schäpe, 2000) in conjunction with simple spectral (layer mean / brightness) and topography metrics, using high resolution imagery such as color-infrared (CIR) and Red-Green-Blue (RGB) images. This model aimed to accurately predict the plant performance of *R. ficaria* populations along the river valley according to a number of input classifications. The model was ground truthed via an observational field study to evaluate the accuracy and effectiveness of the generated model. I expected to achieve greater accuracies predicting high performance plots. I also expected more accurate predictions for plots closer to the river, where soil fertility and species richness appeared to be lower.

### 3.3 METHODS

A variety of different GIS and RS programs were used in combination in order to complete the classification of the Rocky River Valley. Definiens eCognition (Developer 9) was the primary program used to follow the OBIA approach to create the plant performance model. OBIA was used due to the superior accuracies that have been documented through use in modeling projects with similar aims to this project (Batz and Schäpe, 2000; Rego and Koch, 2003). eCognition was chosen due to its strength in large scale image analysis. This powerful program allows the user to develop rule sets for object-based image analysis. eCognition is able to translate a mental model, into computer understandable code and apply an image analysis routine to a multitude of images (Batz and Schäpe, 2000). A mind model involves identifying distinctive characteristics of things that you wish to extract via aggregating individual pixels into meaningful groups, called objects. Rules are then applied based on the defining characteristics of the objects you are trying to classify, such as shape, size or color. For example, to distinguish between rivers and lakes, you would use your knowledge to inform the classification algorithms that rivers are long and thin objects in comparison to lakes which are compact. The OBIA approach necessitates a segmentation of the image to take place, which is adaptable to the scale of interest depending on project aims (Batz and Schäpe, 2000). In order to carry out the plant performance model on Rocky River, rules based upon observational study and the literature of *R. ficaria* were entered into eCognition and applied to imagery. These rules are mathematical interpretations of the current understanding of what site characteristics and imagery patterns would be most helpful in identifying *R. ficaria* populations. These rules rely on knowledge of the species and geometric patterns in order to set up image analysis parameters that can reliably

classify image objects according to the likeliness of *R. ficaria* occurrence and performance. Using characteristics that particular land types have in common, such as pixel area, border index, roundness or color signatures, eCognition is able to classify image objects. For example, river pixels will be black in color, and the length to width ratio will be large. Using this knowledge, these rules will allow eCognition to classify image objects fitting that mathematical description as “River”. As site characteristics are being used to classify *R. ficaria* performance rather than a color signature for example, the model’s predictive power is limited. This was justified as spectrum identification could not be done due to the precise time of year required for the images to be taken was not met. Rules used to identify *R. ficaria* were based upon slope angle, aspect and soil type. Additional GIS software was used, which included ArcMap (10.3.1), ERDAS Imagine (15.0, build 212), RiverTools (4.0.2), Quick Terrain Modeler (8.0.4) and ENVI (IDL 8.4.1, build id: 20150217), among others.

*Sources of Geospatial data:* Digital Elevation Model (DEM) files were retrieved online from the Ohio Geographically Referenced Information Program (OGRIP) website, through the Ohio Statewide Imagery Program (OSIP).

*Preparation of necessary layers:* The layers that were used for the model include; a high resolution color-infrared (CIR) and Red-Green-Blue (RGB) Unmanned Aerial Systems (UAS) image, Digital Elevation Model (DEM), Normalized Digital Surface Model (nDSM), Rocky River raster file (only 7<sup>th</sup> order stream), soil type raster file, slope raster and an aspect raster file. All the layers were subset and clipped to the same dimensions and were reprojected to the same coordinate system (UTM GRS 1980

NAD83 North- Zone 17). The CIR and RGB were resampled to 1 meter pixel sizes, and the rest of the layers were resampled to 10 meter pixel sizes.

The CIR and RGB files were prepared using ERDAS. The CIR was originally obtained off the OGRIP website (OGRIP, 2016, <http://ogrip.oit.ohio.gov/>) and the RGB was originally collected from the National Agriculture Imagery Program (NAIP). Both images were layer stacked, then subset and chipped from the larger files and reprojected accordingly. The DEM was composed in ERDAS using downloaded files from the OGRIP website (OGRIP, 2016, <http://ogrip.oit.ohio.gov/>) to encompass the entire Rocky River. The nDSM was prepared using QT Modeler, and RiverTools was used to extract the Rocky River vector file for use in eCognition.

A soil raster image was retrieved from the United States Department of Agriculture (USDA) through the Natural Resources Conservations Service (NRCS), which was used to represent the various soil types along the Rocky River. The map was cut to include just the three main floodplain soil types, namely Tioga (Tg), Chagrin (Ch) and Euclid (EuA). Rank numbers were attributed to each soil type based on expected *R. ficaria* from 1 to 3 respectively (1 representing the most productive soil) for use in the eCognition classification. This order was selected through evaluation of the various soil characteristics that were found to influence *R. ficaria* performance from the literature and from Chapter 2. Soils of higher pH and moisture (based on soil maps generated by USDA soil surveys) were given a lower rank number as performance increases with increasing soil pH and moisture (Sidari et al., 2008, and Axtell et al., 2010). Further slope and aspect maps were created from the DEM using ERDAS for incorporation into the model. Soil

texture, organic matter and slope angle were additional variables used to aid in ranking, yet these supported the pH and soil moisture recommendations of rank priorities.

*Classification process using eCognition:* A multiresolution segmentation (scale parameter 3, shape = 0.5, compactness = 0.5) was run followed by a spectral difference segmentation (maximum spectral difference = 5). The segmentation refers to the process of dividing the image up into similar groupings of individual pixels and the scale parameter refers to the average image object size. This is a bottom-up approach as it focuses on the pixel scales first, and then extends upwards to the identification of explicit objects.

The next step involved classifying the primitives, which are the basic land type classifications, onto which the plant performance classification will be described. The land types that were classified include, background, water, river, barren land, buildings, pavement, floodplain, grass, shrubs and trees. The primitives primarily relied on the use of the sample editor for classification, which allowed for the selection of multiple samples throughout the image, displaying the characteristics unique to each land type for efficient classification. These characteristics that the sample editor finds to be uniquely characteristic of a particular land type are then used to define each land type accordingly, thus categorizing image objects of similar characteristic signatures as a particular land type. For example, the elevation and reflectance signature unique to grass can be used to distinctly classify grass from trees. As some objects share similar characteristics, a probability of classification is assigned to each category that has been created, and the classification is assigned based on which category has the highest probability.

After the basics had been classified, *R. ficaria* performance (reproductive output and biomass) was then classified using three categories, namely high, medium and low, which were set up using the map layers that had been collected. The maps and imagery that were factored into the model included the soil type, the slope angle, and slope aspect. Certain pertinent criteria were selected for each image layer according to their hypothesized influence they have on *R. ficaria* populations and performance. Evidence suggests that slope can play a major role in driving plant performance and invasion speed as the soil composition can differ substantially (Bragg and Hulbert, 1976). *Ranunculus ficaria* is better suited to soils that have a more gradual slope, as soil properties can vary substantially according to aspect in regions that experience the same vegetation, climate and parent material (Sidari et al., 2008). While no study has directly observed the influence of aspect on *R. ficaria*, performance is expected to be greater on south-facing slopes, where there is increased enzyme activity, organic matter and microorganisms that can be attributed to aspect-induced microclimate differences (Sidari et al., 2008). Thus the parameters selected for the algorithms are based on the best knowledge of expected influence on *R. ficaria* and are designed to classify floodplain areas based on an average of these image layers. Following a successful methodology that has achieved high accuracies in classifying understory shrubs (Franklin et al., 1994), three classes for *R. ficaria* cover (low, medium and high) were distinguished as a means of representing plant performance throughout the Rocky River Reservation. The algorithm for high performance targets lower gradient, south-facing slopes on soil of higher pH, whereas the low performance algorithm favors higher gradient, north-facing slopes on soil of lower pH. These targets are mathematically entered by selecting the probabilities of occurrence



based on the values of each image layer. These mathematical rule sets, also called fuzzy sets, allow elements to have degrees of membership. For example, the slope angle image layer allocates each pixel a value, representing the slope angle in degrees. A membership function (probability curve) for slope angle is entered for each category of *R. ficaria* performance (low, medium and high) based on the best understanding of how that variable drives plant performance. This would mean that a pixel with a low slope angle value would have a high probability of being categorized as high performance. As the slope angle value increases, implying steeper slopes, the probability that a pixel is classified as high performance will decrease (assuming that the other input layers are ignored). All three layers were averaged to give an overall probability of categorization for each pixel. The plant performance categories were restricted to pixels within the floodplain, defined by a buffer area around Rocky River.

The membership functions were adjusted to produce more evenly distributed output based on the three category approach that has been used successfully in the past to achieve high accuracies (Franklin et al., 1994). Evidence based on the accuracy of classifications similar in nature to this project have shown finer segmentation to produce higher accuracies, which aided my decision to use a very fine scale parameter of 3, while keeping shape and compactness at default values of 0.5 (Michez et al., 2016). A scale parameter sets the maximum allowable heterogeneity for image objects. A lower number relates to smaller and more homogeneous image objects.

*Field work for Ground-truthing:* In order to analyze the effectiveness of the generated GIS model, *R. ficaria* populations were sampled in June 2016 from thirty sample sites spanning the Rocky River Valley. A thirty meter transect was set up at each site, with

three quadrats established along a disturbance gradient away from the river (0m, 15m, 30m). The total dried *R. ficaria* biomass was collected from a 50 x 50 cm quadrat, while percent cover estimates were recorded using a 2 x 2 m quadrat. Site variables, such as canopy cover, slope face direction, slope angle and PAR were recorded. For a more detailed explanation of the experimental design, refer to Chapter 2.

*Analysis of generated classification:* The plant performance model results were compared to field observations in order to assess the accuracy and reliability of the classification. The field observations were used to group sites into three categories of performance, namely high, medium and low. These groupings were determined using percent cover measured during the large scale collection at the thirty sample sites spanning the Rocky River. Percent cover is more efficiently extracted from aerial imagery than biomass, and thus was the measurement selected to test the accuracy of the generated model. Percent cover would be a more accurate representation from the model, and this coincides with the methodologies employed by studies of a similar nature (Van Lier et al., 2009). Any site with a percent cover lower than 5% was categorized as low. Any site recording a percent cover of 5-25% was categorized as medium and any site that had a greater percent cover than 25% was classified as high. These are the same categories (high, medium and low) used by the GIS model, and the number of correctly predicted categories based on the classification was used to evaluate the accuracy of the GIS model. Accuracies of predicting each performance level (high, medium, low) and plot distance from the river were recorded and chi-squared analyses were used to test the hypotheses to determine if high performance plots or those closer to the river were most accurately predicted. The chi-squared analyses would show if there are any strong trends

between prediction accuracy and plot types, or alternatively if the observed values do not differ significantly from expected values.

### 3.4 RESULTS

The object-based image analysis model generated a classification of Rocky River in eCognition, predicting plant performance of *R. ficaria* according to three categories; high, medium and low (Figure 3-2). These predictions were informed by aspect, slope and soil type map layers, which were generated in ERDAS (Figure 3-1). Figure 3-2 displays an aerial image of Rocky River in the Lakewood area in Cuyahoga County, Ohio. This aerial image has been classified in eCognition, with sixty-one of the ninety sample plots predicted correctly by the plant performance model, resulting in an accuracy of 68%.

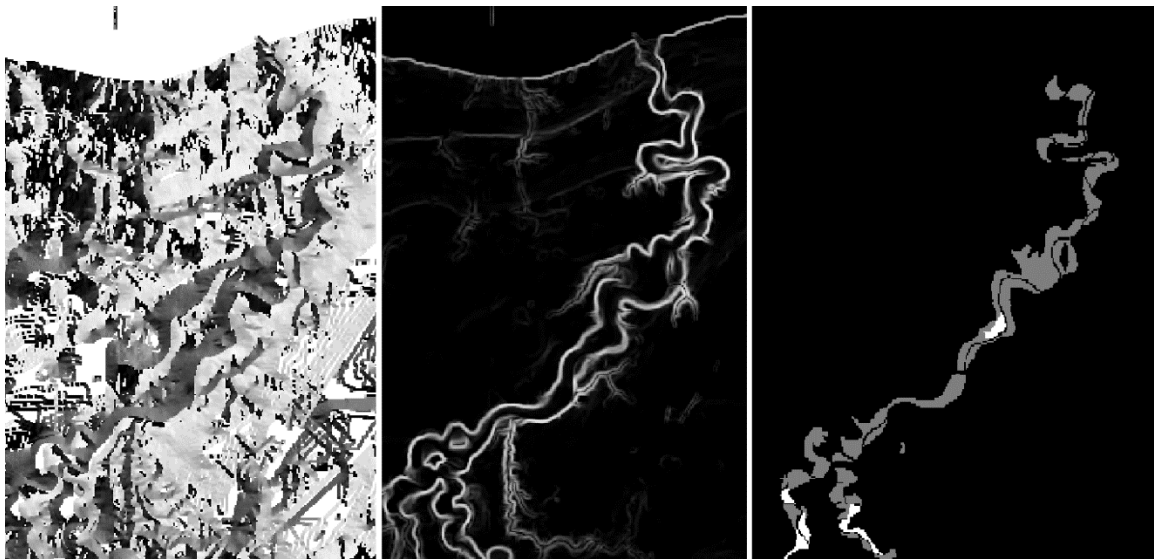


Figure 3-1: Three primary map layers used in the classification of Rocky River, namely aspect, slope and soil type respectively. These layers were developed in ERDAS, and then used by eCognition to predict *R. ficaria* performance along Rocky River.

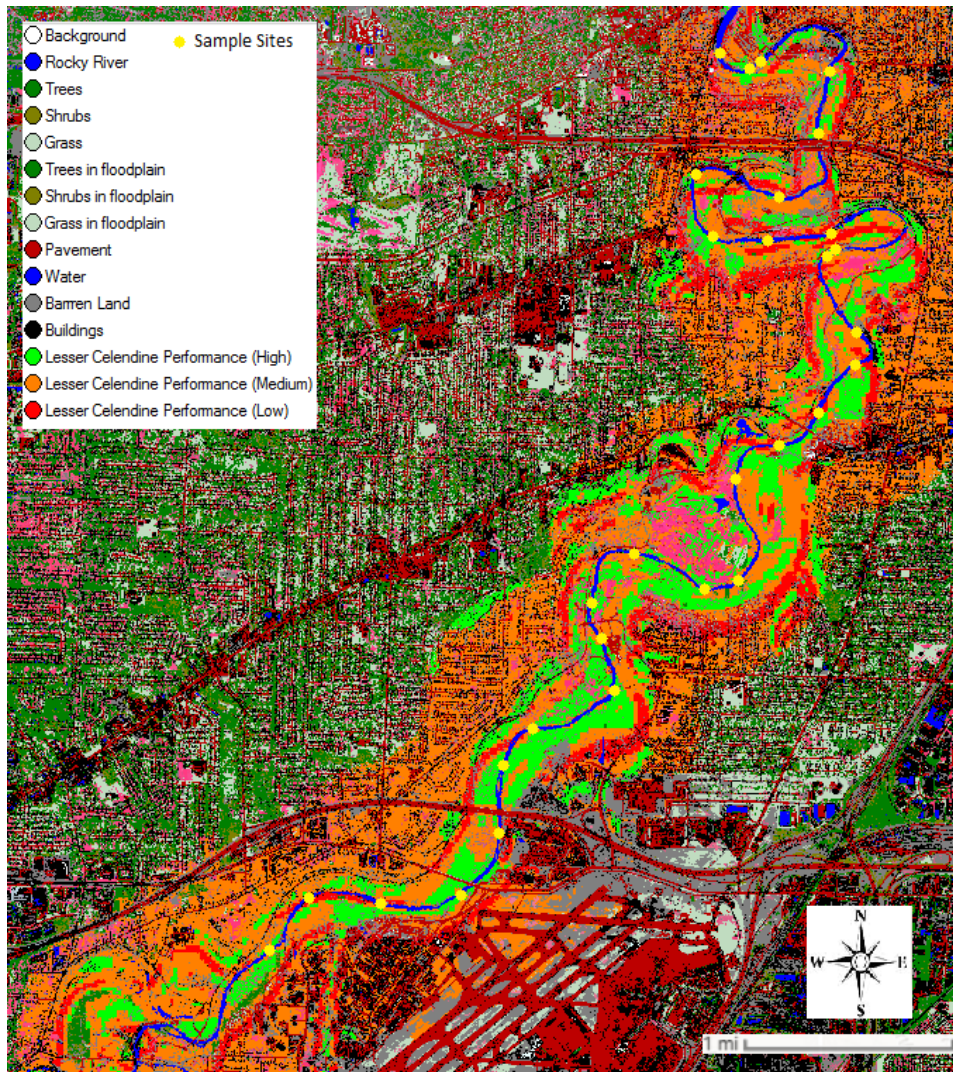


Figure 3-2: Classified image of Rocky River according to predicted plant performance using eCognition. Thirty sample sites along Rocky River were examined based on *R. ficaria* performance predictions according to high (green), medium (orange) and low (red). The overall accuracy for this model was 68%, as tested against fieldwork collections.

The results from the large scale collections in 2016 were used to ground-truth the model, with inaccurate predictions marked in Figure 3-3. The figure depicts all thirty randomly selected sample sites along Rocky River (ninety plots in total), which are color coded according to the plant performance based off percent cover as described in the methods. Out of the ninety plots, twenty-nine were not predicted correctly by the model.

The inaccurate predictions are evenly distributed throughout Rocky River. Small scale variation in quadrat sampling can cause discrepancies between predictions and fieldwork.

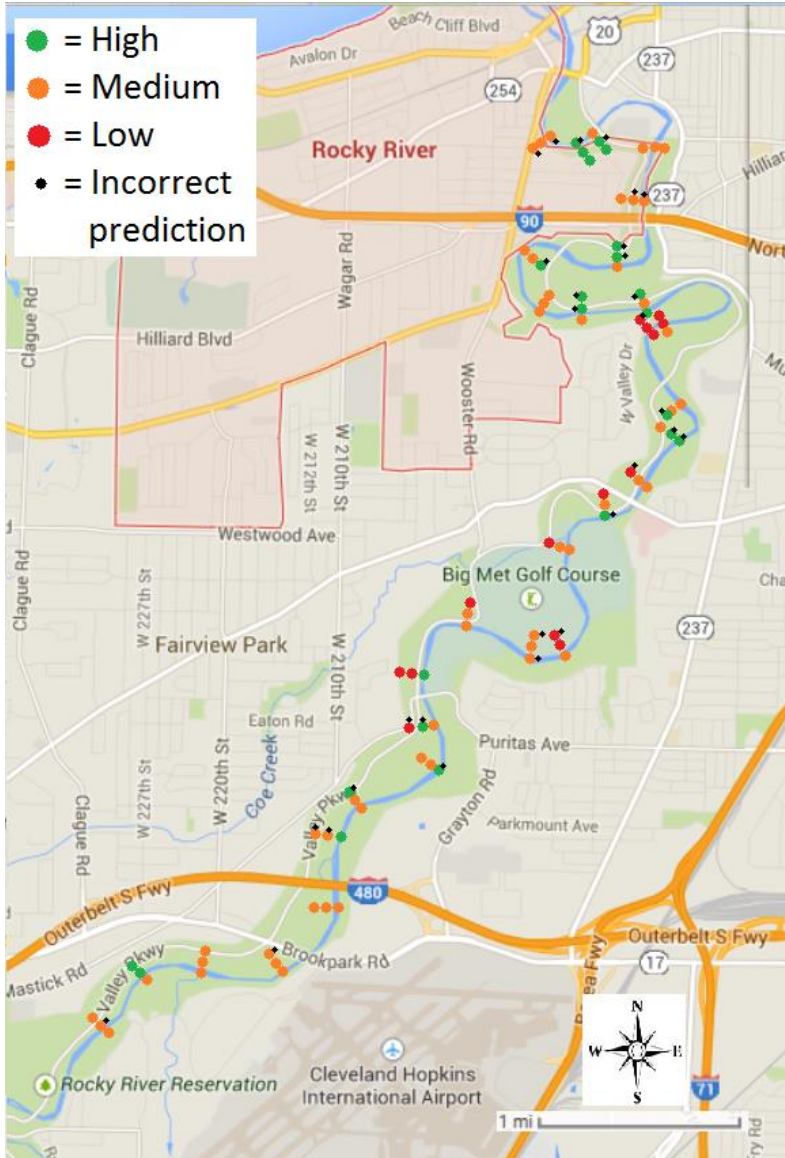


Figure 3-3: Color coded image of the ninety sample plots (thirty sites) according to percent cover as measured during the ground-truthing fieldwork in 2016. Sites where the model incorrectly predicted plant performance are marked with black dots, and these appear to be uniformly distributed throughout the Rocky River Reservation.

Upon completion of the plant performance model, the percent cover measurements taken from the thirty randomly selected sites (ninety plots) were used to test the accuracy

of the generated model. Table 3-1 shows the accuracies of the predictions that the GIS model made for each plot according to performance category and plot distance away from the river. Overall, the model accuracy was 68%. It appeared that medium performance sites were predicted with the greatest accuracy (80% correct), while the most errors occurred with classifying high performance sites (46% correct). Prediction accuracy was significantly different to expected values for plots categorized according to performance. The trend showed reduced prediction accuracy for high performance plots. This was the opposite of what was expected; therefore I cannot support my hypothesis (Chi-Square,  $X^2_3 = 7.930$ ,  $P < 0.025$ ). Plots closer to the river appeared to be most accurately predicted (77% correct), while prediction accuracy seemed to decrease with the distance of plots from the river. Prediction accuracy however was not significantly different for plots categorized according to their distance from the river; therefore I cannot support my hypothesis (Chi-Square,  $X^2_3 = 1.406$ ,  $P > 0.1$ ). This implies that the observed accuracies were not significantly different from the expected accuracies, suggesting neither characteristic was a reliable predictor of model accuracy.

	Wrong	Correct	Correct (%)
High	7	6	46
Medium	11	44	80
Low	11	11	50
Total	29	61	68
Accuracy (%)	<b>68</b>		

	Wrong	Correct	Correct (%)
Q1	7	23	77
Q2	8	22	73
Q3	14	16	53
Total	29	61	68
Accuracy (%)	<b>68</b>		

Table 3-1 a and b: Contingency tables evaluating the accuracy of the plant performance model based on fieldwork results from 2016 collections as a comparison. (a) Evaluates the prediction accuracy based on performance categories and (b) evaluates accuracies based on plot distance from the river. Overall the model accuracy was 68%.



*Ranunculus ficaria* has not been mapped before using the OBIA approach. The accuracy of the generated model is similar to studies involving other understory invasive species. A wide spectrum of accuracy was recorded during a study aimed at mapping three riparian invasive plants using the same methodology. Accuracies for *I. grandulifera*, Japanese knotweed and *H. mantegazzianum*, were 72%, 68% and 97% respectively, which displays the variability of mapping by species (Michez et al., 2016).

### 3.5 DISCUSSION

This OBIA plant performance model of *R. ficaria* composed in eCognition yielded an accuracy of 68%, which is reasonable for the prediction of an understory plant species and falls in the range of models of a similar nature. There were no significant trends between prediction accuracy and distances away from the river. There were significant differences between the expected and observed accuracy of plots categorized by plant performance, with the greatest accuracy achieved for low performance plots. This trend opposes my hypothesis and therefore I cannot support either hypothesis. Beyond selecting a different target species, there are available options for increasing the model accuracy, such as incorporating higher resolution imagery and having a greater number of field sites to analyze had time allowed. Overall the OBIA methodology is a desirable and time effective approach that can be used to aid the decision making of land managers. The greatest challenge to achieving a greater accuracy was the target species; *R. ficaria*, being an understory species, which can be difficult to map (Joshie et al., 2004).

OBIA has produced results for similar models assessing habitat suitability, using its bottom-up methodology (Batz and Schäpe, 2000; Rego and Koch, 2003). Using OBIA methodology itself has been identified as a potential method for increasing accuracies by

around 10%. This was a strong driving force behind using it for this study (Hantson et al., 2012). Another study in South Africa found that using OBIA instead of common pixel-based classification increased the overall mapping accuracy from 78% to 90% (Gairola et al., 2016). Similar projects have had variable success, with accuracies in the range of 40% to 99% depending on the species, methodology and resolution of imagery used (Franklin et al. 1994; Van Lier et al. 2009; Wilfong et al., 2009; Michez et al., 2016).

There were many limitations to the model that could have influenced the accuracy achieved. Evidence shows a wide range of accuracies that can be achieved based purely on species of interest, where the same methodologies have been applied (Michez et al., 2016). Understory species are vastly more difficult to map with high accuracy, and this is evident in the GIS literature as a large majority of GIS and RS studies have been applied to canopy dominant species, despite the overwhelming majority of invasive plants being understory species (Joshi et al., 2004). This implies that when the methodology is kept constant, accuracies may be highly variable depending on the species of interest. The addition of elevation can aid the success of understory species mapping, for which Tuanmu et al. (2010) showed that the addition led to an increase in understory plant accuracies for two bamboo species (*Bashania faberi* and *Fargesia robusta*) from 59% and 89% to 68% and 91% respectively. This led to the inclusion of elevation data in the classification of floodplain vegetation types for increased accuracy. Slope gradient would likely further add to the accuracy achieved, especially considering the negatively significant relationship between slope and *R. ficaria* biomass and percent cover (Chapter 2).



Another variable that influences model accuracy is the spatial resolution of the images used. Studies have found that while using lower resolution imagery can be a cost-effective way to map invasive species, the inclusion of higher resolution imagery generally achieves higher overall accuracies (Franklin et al., 1994, Van Lier et al., 2009; Hantson et al., 2012). While this study made use of some high resolution imagery (RGB and CIR, 1 m pixel size), if the additional DEM based layers were higher in resolution (LiDAR), accuracies could have been expected to increase by around 10% (Hantson et al., 2012). Unfortunately due to difficulties manipulating the LiDAR images, they were unable to be included in the classification. Franklin et al. (1994) demonstrated that the classification of plant cover into three categories, similar in methodology to this study, could achieve high accuracies of between 87% and 99% when using high resolution imagery. Van Lier et al. (2009) mapped the same species of ericaceous shrubs using imagery of different spatial resolutions, namely IKONOS and Landsat, and achieved accuracies of 87.7% and 79.4% respectively.

While additional map layers could be considered, simple spectral data is most useful for the classification of various land cover types (Joshi et al., 2004). If a more detailed soil map existed for Rocky River, preferably mapping soil pH, incorporation could have led to more accurate predictions. The incorrect predictions could be attributed to high variability at small scales in the field, where small differences in distance for transect setups could lead to drastically different results, and thus site classifications. The incorrectly predicted sites are uniformly distributed along the river and therefore do not show any trends related to the area along the river. Distance away from the river does appear to play a role, with greater accuracies recorded for sites closer to the river (77%).

As canopy cover and species richness appears to increase with distance away from the river, this may add additional interactions and complexity making it more difficult to accurately predict *R. ficaria* infestations. Fieldwork from Chapter 2 supports this, as the variability for many response variables is lower, closer to the river.

The underlying methodology of administering the OBIA approach to inform management is promising. Similar projects have achieved high levels of accuracy, especially using canopy dominant species. Plant species play a substantial role in explaining model accuracy, and while 68% does not reach the accuracy of many models, it is a reasonable result for an understory species. The modeling accuracy of *R. ficaria* would be expected to increase as the body of scientific literature on this invasive ephemeral grows, due to better informed models. This model, in conjunction with results from Chapter 2, shows elevated *R. ficaria* performance on low gradient, south-facing slopes that have high pH and soil moisture content with low variability. Instead of applying management efforts uniformly across invaded populations, the limited resources should be focused by land managers on sites matching these characteristics. This would help to optimize management by means of the targeting of more critical and risky populations of *R. ficaria*, where higher performance and more rapid spread are expected. Spread is expected to be greater at sites with higher plant performances, especially when coupled with *R. ficaria*'s early growing season which allows it to spread and crowd out native competitors before they emerge.

Being able to accurately map high performance sites would be extremely valuable for land managers, as this would illuminate critical infestations to target. It was hypothesized that high performance plots may be easier to predict, because it is often more difficult to

find commonality in failed invasions, yet the results do not support this hypothesis. There was a significant trend observed between accuracy and plot performance, with greater accuracy being achieved for lower performance plots. High performance sites actually achieved the lowest accuracy (46%). Chapter 2 suggests that soil is more fertile further from the river, with increased calcium levels, pH and soil moisture. High performance plots were more commonly found at further distances away from the river. This may lower the accuracy of prediction due to the greater uncertainty linked to increased complexity and species richness. The results show that observed accuracies were significantly different from what was expected and therefore suggest that plant performance may be a reliable predictor of model accuracy.

This plant performance map has added to our general understanding of *R. ficaria* population dynamics through confirming the influence that soil, slope and aspect may have on plant performance. Plots closest to the river were the most accurately predicted at 77%, yet analyses found no significant trends between accuracy predictions and plot distance away from the river. This suggests that observations were not significantly different from expected accuracies, thus I cannot accept my hypothesis. Plot distance away from the river did not allow for reasonable predictions of model accuracy.

Overall, this OBIA model for *R. ficaria* was reasonably accurate in predicting plant performance and this method could be used by land managers for early detection and prioritization of management resources. Management suggestions would be to focus attention on high risk populations where soils are fertile (high pH and nutrient content) and slopes are gradual. This project demonstrates the potential for a cost-effective, practical approach towards management prioritization. GIS and RS software have proven

to be extremely powerful tools that can be used to help land managers make informed decisions with regards to optimizing the management of invasive species.

## CHAPTER IV

### CONCLUSION

#### 4.1 CONCLUSION

In summary, the extensive harm that invasive plants can cause on a global scale has been well documented by ecologists. They threaten native communities, ecosystem services and biodiversity, along with a host of additional environmental and economic consequences (Jose et al., 2013; Pimentel et al., 2005; Whitfeld et al., 2014). Invasive species are not always problematic across all environments, and identifying critical populations is imperative for effective management as land managers have limited resources (Rew et al., 2005). Variation in invasiveness occurs according to environmental and species specific influences (Rew et al., 2005). Greater understanding of an invasive, along with early recognition grants an opportunity to more efficiently manage a particular species, and if already widespread, efforts can be focused on minimizing further spread (Hobbs and Humphries, 1995). *Ranunculus ficaria* L. is a major species of concern to

land managers in Ohio, for which a greater understanding is necessary to mitigate environmental problems. *Ranunculus ficaria* can cause extensive environmental impacts especially in floodplain lowlands due to its early growing season, high reproductive output and vegetative growth patterns (Axtell et al., 2010). This project aimed to shed some light on the environmental driving forces behind this invasive perennial, adding to our currently limited understanding in hopes to aid management efforts. This chapter aims to summarize key findings from previous chapters, and to identify potentially fruitful future directions that may help to fill in some of the gaps in the sparse *R. ficaria* literature.

This research project was partially supported by an Ohio Invasive Plants Council (OIPC) Research Grant (2014), which is a non-profit, Ohio-based coalition of organizations that is concerned about the control and spread of invasive plants. The OIPC the aims to preventing new invasions in Ohio through the promotion of awareness of invasion issues, while aiding land managers and researchers increase efficiency where possible (OIPC, 2015). In order to alleviate the conflicts between management and industry, the OIPC has been working with industry with aims of mitigating environmental damage and a greater understanding of harmful species should relate to more beneficial outcomes. As *R. ficaria* has been identified as an invasive species of high concern, both the OIPC and the Cleveland Metroparks have wanted to know more about this invasive perennial.

This project has contributed to the limited body of knowledge on *R. ficaria*, identifying many interesting influential site characteristics that could be driving populations. Soil characteristics were the most influential factors related to increased

plant performance, especially soil pH, texture, moisture and nutrients (P, Ca) where soils of greater pH, moisture, percent silt and soil nutrients were found to have healthier, more productive populations of *R. ficaria* according to biomass and reproductive outputs (Chapter 2). Other influential site characteristics that were identified during the large scale collections were slope gradient and canopy cover, where more gradual slopes, with greater canopy cover were found to have significantly greater *R. ficaria* biomass and percent cover (Chapter 2). A plant performance model was generated using eCognition software, which explored how technology can be used as a cost effective method to aid management prioritization (Chapter 3).

This information can be helpful in aiding management decisions related to *R. ficaria* through adding to the collective understanding of what factors may be influencing plant performance. Due to the importance of soil characteristics found in Chapter 2, management should be prioritized based on critical areas of favorable conditions rather than blanket treatment. In Rocky River, the prolific spread of *R. ficaria* has led to large scale infestations that can be difficult to eliminate especially at sites where the whole understory is overwhelmed by *R. ficaria*. Where this is the case, management should focus efforts on reducing the spread by identifying suitable surrounding environments where *R. ficaria* can be excluded from. Suitable areas would likely have gradual floodplain slopes, with soil that is high in moisture content, pH and nutrients such as phosphorus and calcium. Plant performance is greater on soil with high percent silt, which contains higher levels of calcium. In less invaded areas, soil and elevation maps could be used to prioritize certain populations for management or predict critical areas that may be suitable and susceptible to *R. ficaria* invasion in future. The GIS based plant

performance model was able to demonstrate an efficient methodology that could be used to remotely aid management decisions, which would be especially valuable due to limited management resources, exacerbated by the small critical window period for management of this harmful species.

There is still much to learn about this invasive buttercup, thus there are plenty of opportunities for further research and experiments that would be helpful. Although germination appears extensive in the field, bulbil and tuber germination has been unsuccessful under controlled conditions in the greenhouse even after cold stratification for varied lengths of time. Better understanding bulbil dormancy would be an extremely important step towards understanding this troublesome invasive as it would open the door to an extensive range of greenhouse experiments that could be set up. With such poor control over the germination of this species in the greenhouse, one cannot set up any rigorous experiments yet. Beyond the bulbil dormancy, there are still many opportunities to investigate plant-animal interactions, seed production and fertility as well as further investigation into dispersal mechanisms of this invasive buttercup. Other forces may also be pertinent in describing the population spread of *R. ficaria*, thus additional variables could also be investigated such as flooding interactions or climate change influences. Additional locations or studies of a longer time frame might also prove valuable to further increase our understanding of this invasive perennial. Overall, this study was able to add to the *R. ficaria* literature by the identifying influential site characteristics behind the successfulness of this herbaceous invasive and thus inform management decisions.



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APPENDIX

A. Historical Climatological Data Analysis:

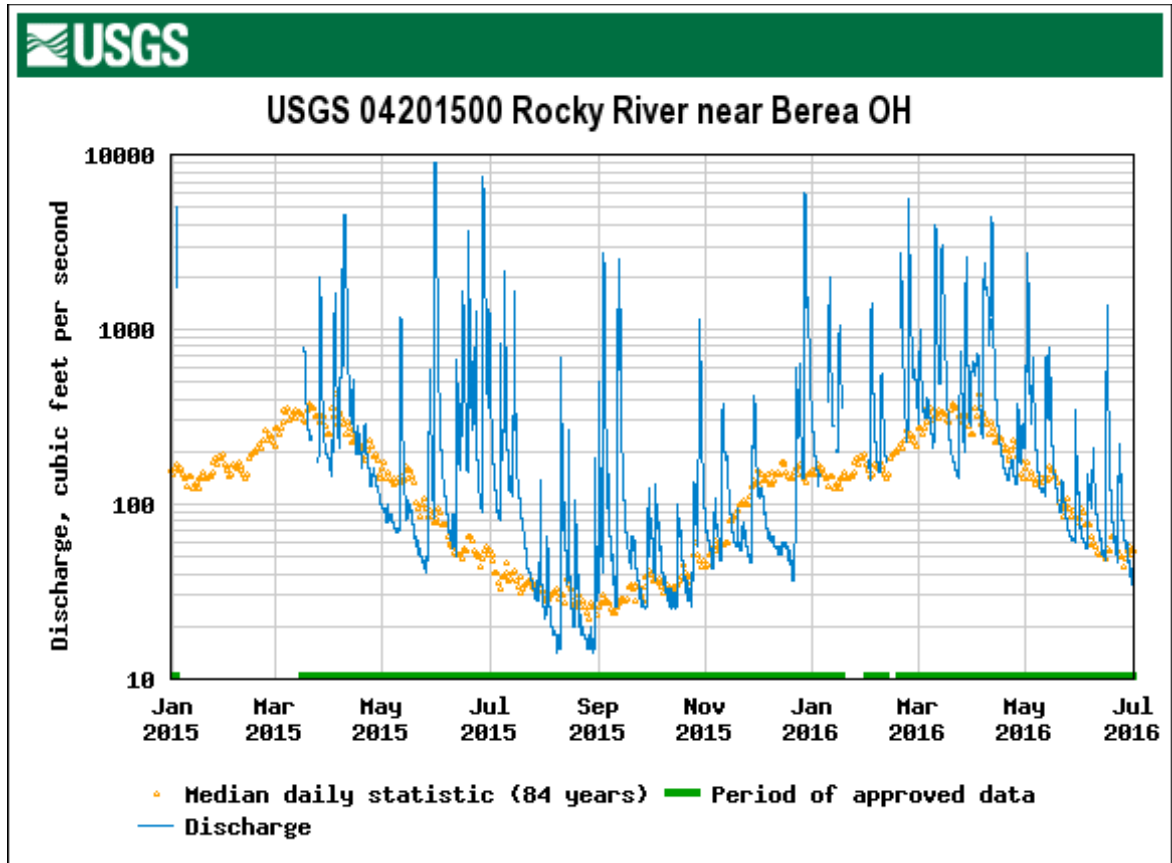


Figure A-1: Historic discharge data recorded along Rocky River from January 1<sup>st</sup> 2015 up to July 1<sup>st</sup> 2016, retrieved from [nwis.waterdata.usgs.gov](http://nwis.waterdata.usgs.gov).



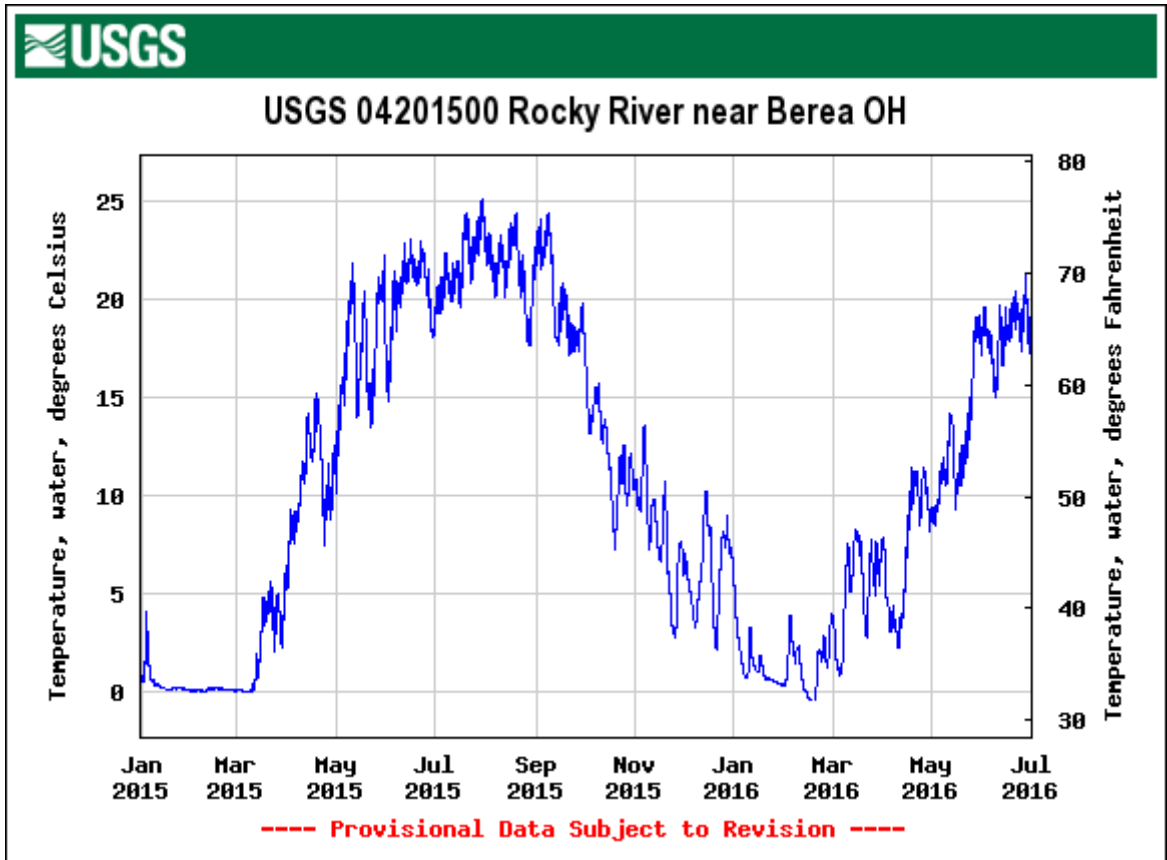


Figure A-2: Historic water temperature data recorded along Rocky River from January 1<sup>st</sup> 2015 up to July 1<sup>st</sup> 2016, retrieved from [nwis.waterdata.usgs.gov](http://nwis.waterdata.usgs.gov).

When looking at the discharge recorded for the Rocky River area near Berea, the starkly different weather conditions experienced during the two year period are noticeable. Weather can play a major role in dictating field observation patterns in multi-year data collections. The 2015 winter was much colder and displayed a greater amount of precipitation with longer periods of snow and ice cover. This is evident when looking at the discharge with just over three months where no data was recorded, starting January 2015 (Figure A-1). This lack of discharge data represents ice cover, and this period extends for a much lengthier time than the 2016 year where the river appeared to generate data for most of the winter and spring, barring a small cold spell that can be seen in the late January of the 2016 temperature data (Figure A-2). The 2015 field season saw

increased variation once data resumed after the ice melt, with periodic surges and dry spells that occur for longer periods than those observed in 2016. The discharge from June 2015 shows data well above the median daily statistic, after a series of low discharge weeks. The 2016 discharge is more consistent and deviates less from the median daily statistic in comparison to the erratic 2015 field season. The temperatures of the river at various times during the spring are interesting to note, especially with the extended 0 degree record from January to past March of 2015. The low temperatures persisted for a long period of time, yet in 2015 the temperature of the water reached 15 degrees Celsius during April, whereas in 2016 it took almost up until June to reach that same temperature. By this time in 2015 the water temperature was seen to be fairly consistently above 20 degrees. This is a large difference as the cold winter rebounded sharply into a warmer spring to summer transition. This time period in 2016 showed a mild winter with slow warming in comparison to 2015 and this can be observed in the steeper curve for the 2015 growing season (Figure A-2).

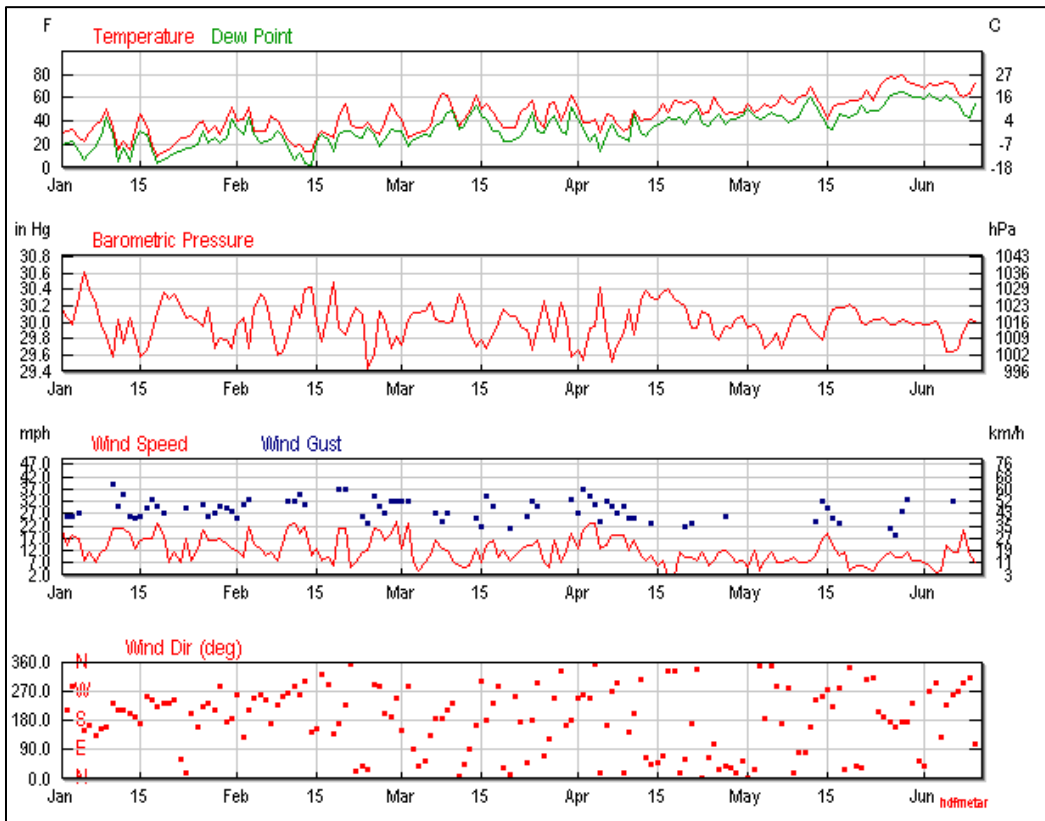
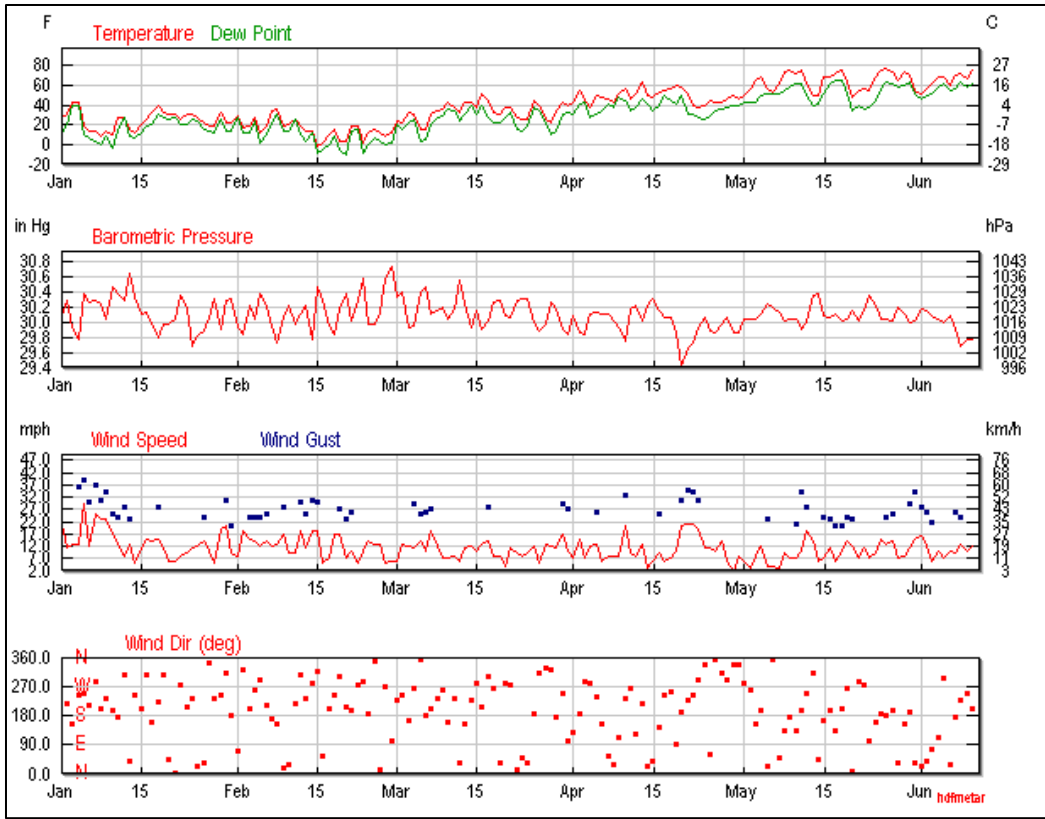


Figure A-3: Showing the historic temperature, pressure, wind speed and direction records from February 1<sup>st</sup> to June 10<sup>th</sup> for 2015 and 2016 respectively. Data was retrieved off wunderground.com for Cleveland Hopkins International Airport.

The 2016 field season showed a lower plant biomass and higher density than the 2015 field season. The *R. ficaria* biomass was marginally lower, yet the density was almost double. The biomass from 2015 may be higher than 2016 due to site 1 having exceedingly high biomass results that could potentially shift the mean. The individual bulbil counts were also almost double in 2016. This seems to indicate healthier populations under the higher winter and spring temperatures, as well as the elevated precipitation. It is interesting to compare and contrast the temperature data for 2015 and 2016. The 2015 winter was significantly more severe, with temperatures reaching much colder levels with greater amounts and sustained snow cover. The first stand out when looking at figure A-3, is the different scales used in recording temperatures, where an additional 20 degrees below 0 was needed to fully display the full range of 2015 temperatures. The 2016 spring cooled off, whereas the 2015 spring consistently increased in temperature after the snow melted. The temperature of the water during the 2016 growing season generally lagged behind that of 2015, which coincides with the growing degree days in 2016 being rapidly caught and overtaken during the spring field season. There appears to be a large amount of variation in the data collected as the bulbil counts per stem and the tuber counts were lower in 2016. There are many limitations that can be observed from this two year study, with a wide range of variables interacting in a complex community setting. When looking at spring ephemeral communities over a two year period, there can be large variations attributed to significantly different winters and trends may be more enlightening with

additional years of data collection. Cleveland experiences wildly different winters, making it important to consider these variations when analyzing data.

### *B. Greenhouse Germination Experiments*

Five germination experiments have been conducted in order to investigate viability and germination cues, using bulbils collected from Rocky River at different times, and cold stored for varied lengths of time. Despite the rapid growth and consistent germination observed in the field, it was surprising that both the bulbils and tubers failed to germinate under controlled conditions. In all five of the experimental trials there was zero germination observed (Table 1). This implies that the necessary conditions to break bulbil and tuber dormancy were not met in the greenhouse setting, despite varied lengths of cold stratification and soil types.

The literature suggests that cold stratification for longer than 2 weeks at 4 or 8 °C is optimal to induce tuber germination (Kertabad et al., 2013). Alternative methods and conditions need to be explored in order to unlock the secret of dormancy observed in the bulbils and tubers of *R. ficaria*. The timing of bulbil and tuber collections and planting may play an important role in the germination success or lack thereof. The greenhouse is also technically limited, and thus the ambient room temperature and sunlight availability may have had an impact. In addition to cold stratification, gibberellic acid, scratching, altering day/night regimes, as well as differing the temperature ranges and osmotic pressure could also be investigated in the hopes of inducing germination.

<b>Trial #</b>	<b>Collected: Date + Site</b>	<b>Cold room Date + Length</b>	<b>Date Planted</b>	<b>Soil Type + Depth Planted</b>	<b># of Bulbils/ Tubers Planted</b>	<b>Germination Before Planting?</b>	<b>Observed Germination or Growth</b>
1	October 4 <sup>th</sup> , Site 3	-	October 9 <sup>th</sup>	Potting, varied	16 x B, 8 x T	-	0% germination
2	October 4 <sup>th</sup> , Site 3	October 20 <sup>th</sup> , 25 days	November 14 <sup>th</sup>	Potting, surface	20 x B	-	0% germination
3	October 4 <sup>th</sup> , Site 3	October 20 <sup>th</sup> , 28 days	November 17 <sup>th</sup>	MP (site 2), surface	21 x B, 5 x T	-	0% germination
4	December 6 <sup>th</sup> , Site 6	December 7 <sup>th</sup> , 5 days	December 12 <sup>th</sup>	Potting, surface	15 x B	10 had germinated	No growth + 0% germination
5	October 4 <sup>th</sup> , Site 3	October 20 <sup>th</sup> , 3.5 months	January 27 <sup>th</sup>	Potting, surface	12 x B	-	0% germination
6	January 10 <sup>th</sup> , Rocky River + golf course	-	January 27 <sup>th</sup>	Potting, surface	12 x B	7 had germinated	No growth + 0% germination

Table A-1: Displays the results of five different germination trials conducted in the greenhouse. All five of the trials resulted in zero germination.