Effective control methods and the genetic and phenetic differences of European dewberry (*Rubus caesius*) among locations in the Finger Lakes Region of New York

By

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A thesis submitted to the Department of Environmental Science and Ecology at SUNY Brockport, in partial fulfillment of the requirements for the degree of

Master of Science

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Thesis Defense

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Abstract

The colonization of invasive species is rapidly increasing due to human travel, trade, and disturbance. An area of focus in invasive species control efforts is in riparian zones, which are often highly invaded and disturbed systems. *Rubus caesius* (European dewberry) is a nonnative invasive woody shrub that has recently been observed to grow densely and spread through riparian ecosystems in western New York. Multiple locations have been noted, but it is not clear if all locations of *Rubus caseius* are correctly identified, as morphological and reproductive characteristics vary among them. *Rubus caesius* is a relatively understudied invasive plant and it is not known how *R. caesius* is dispersing or how to effectively control extant locations. To understand the dispersal mechanisms of *R. caesius* I conducted a greenhouse cutting experiment and quantified the fruiting characteristics among six locations across western New York. I found that *R. caesius* is capable of reproducing via fragmentation regardless of source location, while only some locations produce large numbers of seeds. I investigated location differences through vegetative morphometrics and microsatellite fragment analysis. I found that all locations are identified correctly as *R. caesius* but there is some evidence for differentiation among the locations. Contrary to my expectations the most similar locations are not always the closest geographically. To evaluate effective control measures, I conducted a control experiment in a randomized block design at one located at Ganondagan State Historic Site in Victor, NY. I compared mechanical and herbicide treatments in different combinations of frequency, control method, and herbicide type. Treatments using chemicals were most effective in reducing the cover of *R. caesius* across a variety of environmental conditions. I also provide tentative evidence that repeated mechanical treatments are effective in reducing the cover of
*R. caesius*. My determination of potential dispersal mechanisms and effective control methods will be useful for land managers as management strategies may need to differ based on reproductive traits among locations.

**Keywords:** invasive, *Rubus*, riparian, genetic, phenetic, control
General Introduction

In the spring of 2017, an unknown Rubus species was observed invading the Great Brook stream at Ganondagan State Historic Site in Victor, NY. It was later identified as Rubus caesius by park staff. Rubus caesius is a nonnative invasive woody shrub native to Europe and Asia that was brought to the United States in 1897 (Widrlechner and Wagner Jr. 1998). R. caesius has not historically been considered a threat to the communities it inhabits, but land managers have recently noticed it growing densely and encroaching on sensitive areas. Ganondagan State Historic Site staff were concerned that R. caesius would hinder their efforts to restore riparian habitats. As little is known about effective control of R. caesius, staff from Gandondagan State Historic Site reached out to SUNY-Brockport about conducting an eradication experiment to determine best management practices. We investigated the effectiveness of mechanical, chemical, and the combination of mechanical and chemical treatments. Chapter two reports on these results.

A staff member at Green Lakes and Chittenango Falls State Parks in Syracuse, NY heard about the work being conducted at Ganondagan State Historic Site for R. caesius control as they recently encountered R. caesius to be invading the habitat of the endangered Chittenango Ovate Amber Snail (NYDEC 2022). Upon visiting these sites, we observed that these R. caesius individuals appeared to have different morphometrics than the Ganondagan locations. The Ganondagan location exhibited longer petiolules, a longer terminal leaflet, and fewer fruits (as a single drupe) than the Syracuse locations. We also discovered several other locations of R. caesius in the western region of the Finger Lakes, including a location at the SUNY Brockport campus and another near the Honeoye Creek Wildlife Management Area in
Honeoye, NY. Individuals at these locations were observationally most similar to those at the Ganondagan location.

In the second chapter of my thesis, I investigate differences among six western New York locations by quantifying genetic, phenetic, and reproductive metrics. Morphometric measurements can provide quantitative data to evaluate small differences in phenetics among and within locations (Baret et al. 2003). However, morphology is often affected by environmental conditions, so genetic evaluation is used to supplement morphology (Alice et al. 1999). I decided to use simple sequence repeats (SSRs) as they can determine genetic fingerprints, DNA profiling, and kinship (Provan et al. 2001). Recent work by Petra Sarhanova, who used *R. caesius* as an outgroup in her SSR analysis, provided me with the successful primers she used to undertake this work (Sarhanova et al. 2017). Chapter one reports on these results.
Literature Cited:


Chapter 1

Comparison of the phenetics and genetics of European dewberry (*Rubus caesius*) in the Finger Lakes Region of New York

**Introduction:**

The increasing distribution of invasive species is a significant component of global environmental change (Vitousek et al. 1997). Once present in a community, invasive species can disrupt plant communities and cause long-term impacts on the ecosystem (Mack et al. 2000). It is critical to learn more about the traits of invasive species to slow current and future invasion through conservation efforts (Mack et al. 2000, Trebitz and Taylor 2007). Invasive species establish in new environments after initial dispersal (Kuebbing et al. 2014, Mack et al. 2000). Understanding dispersal mechanisms is one key to conducting proper eradication techniques (Mack et al. 2000).

Many invasive species arrive at new habitats due to biotic dispersal of seeds (Demaio et al. 2011, Sperry et al. 2021). The consumption of fruit by birds and other animals facilitates dispersal farther than previously possible through other means such as wind or water (Cronk and Fuller 1995). This facilitates further invasion throughout ecosystems where it would normally not be able to reach (Lockwood et al. 2005). Additionally, invasive species contain greater number of seeds per fruit than native counterparts (Demaio et al. 2011, Lockwood et al. 2005). Invasive species also have been observed to fruit for a longer period of time than native species; Sperry et al. (2021) found that invasive plant species fruited for three more months than native counterparts. Both of these traits increase the chance of viable
seed dispersal. Seed dispersal by animals is common among invasive plants (Cronk and Fuller 1995, Vergara-Tabares et al. 2015).

Vegetative dispersal through stem fragments is also important for the dispersal of invasive plants. Compared to other means of asexual propagation, fragments propagate easier, have longer periods of survival, and have high colonization and regeneration rates (Riis and Sand-Jensen 2006, Barrat-Segretain et al. 2000). Cultivation experiments such as that performed by Xie et al. (2010) have quantified the propagation and dispersal of invasive plant fragments. Invasive plants have better growth traits than their native counterparts (Xie et al. 2010). These higher growth rates of the invasive species lead to more branch productions, further fragmentation, and therefore further invasion success (Xie et al. 2010, Liu et al. 2006).

Invasive species may disperse primarily through seeds (e.g. *Ailanthus altissima* (tree of heaven)), vegetative fragmentation (e.g. *Nitellopsis obtusa* (starry stonewort)), or both. Some invasive species can exhibit different traits that affect dispersal characteristics among different populations (McDowell and Radosevich 2005). These differences can include different flower and fruit density (McDowell and Radosevich 2005, Dale et al. 2005). There can be differences among populations due to local adaptation, phenotypic plasticity, or different genetic makeup (Richards et al. 2006). Some invasive species have shown the ability to adapt to their habitats by altering their dispersal patterns to include both methods, often through hybridization (Whitney 1984, Alice et al. 1999) This allows for an even more rapid distribution of the species and greater spread. To quantify the differences among populations, morphometric measurements are used.
Morphology is the simplest and most intuitive method for identifying the phenetic differences between populations and among species (Caplan and Yeakley 2013, Laport and Ramsey 2015). Morphometric measurements allow for the quantitative analysis of the external size and shape of a given species (Baret et al. 2003, Laport and Ramsey 2015). Therefore, morphometrics are often used in systematic studies because it gives quantitative data to support the small differences in phenetics among morphotypes (Baret et al. 2003,). These measurements are often used in plant studies because it is both cost effective and efficient in determining specific differences in vegetative, floral, and fruit characteristics among similar species (Billet et al. 2017, Caplan and Yeakley 2013). Although morphological measurement is effective in quantifying these differences, morphology is also easily affected by environment (Laport and Ramsey 2015). Thus, genetic evaluation is used to determine differences among species without the influence of environmental factors (Alice et al. 1999).

On the genomic level, hybridization enhances adaptation through rapid reproductive isolation between hybrid and parental species (Comai 2005, Rieseberg and Willis 2007). This hybridization mostly occurs between species that are closely related, and even subgenera, but can also occur between distantly related species (Alice et al. 1999). The use of molecular markers provides insight into morphometric differences and plant hybridization. Microsatellite markers, or simple sequence repeats (SSR), are often used in determining small genetic differences in the same or closely related species when there appear to be morphometrical differences (Provan et al. 2001). When analyzed, these microsatellite primers produce products that show the variation in the microsatellite primers through different fragment lengths. However, often times the differences between microsatellite
regions are so small, that they cannot be detected by average gel electrophoresis (Provan et al. 2001, Sarhanova et al. 2017). Therefore, capillary electrophoresis (fragment analysis) is used in order to detect these differences in allele length, heterozygosity, and the frequency of alleles (Provan et al. 2001, Varshney et al. 2005).

A common landscape that holds invasive species capable of both seed and vegetative dispersal and hybridization is riparian zones. With reoccurring flood events, the interconnected waterways of riparian zones facilitate rapid seed and vegetative dispersal throughout the watershed (Michez et al. 2015, Richardson et al. 2007). Riparian zones hold a high biodiversity and provide crucial habitat for many plants and animals. However, riparian zones are susceptible to invasive species because they are under continual disturbance through flooding and drought events (Michez et al. 2015, Richardson et al. 2007). Long distance dispersal of the vegetative fragments and seeds leads to further invasion throughout the watershed (Jacquemyn et al. 2006, Richardson et al. 2007).

The *Rubus* genus commonly thrives in riparian habitats due to increased disturbance and further dispersal by flood events (Waugh et al. 1990, McDowell and Radosevich 2005). Many *Rubus* species reproduce through the dispersal of seeds by the consumption by birds and animals. Other *Rubus* species disperse vegetatively by rooting at the tip and forming rhizomes (Waugh et al. 1990). Species such as *Rubus idaeus* have been reported to disperse both through seed and vegetative fragments (Whitney 1984). A study comparing the reproductive differences between invasive and native *Rubus* species found that the *Rubus* species that are able to reproduce both sexually and vegetatively are commonly considered invasive (McDowell and Radosevich 2005). This ability to reproduce both through seed and
vegetative fragments increases the chance of successful reproduction and further invasion (McDowell and Rado

The genus *Rubus* is also one of the most taxonomically challenging genera due to a high degree of morphological diversity and frequent hybridization (Waugh et al. 1990). There is great morphological diversity within the *Rubus* genus as species show a wide range of vegetative, floral, and fruit characteristics (Alice and Campbell 1999). An explanation for such morphological diversity within the genus is hybridization (Alice and Campbell 1999, 2001, Sochor et al. 2015). One species of *Rubus* that often naturally hybridizes with similar species is *Rubus caesius* (Sochor et al. 2015). *Rubus caesius* has been reported to hybridize with related species such as *Rubus idaeus* to form various hybrids that vary in morphology and genetic make-up (Laport and Ramsey 2015). Hybrids have been shown to have an increased number of flowers, larger seed mass, and greater specific leaf area (SLA, Feng et al. 2008).

*Rubus caesius* is a nonnative invasive woody shrub native to Europe and Asia. It was first brought to the United States in 1897 to be used as an erosion control tactic along streambanks (Widrlechner and Wagner Jr. 1998). It is currently found in 10 states. In its native habitat, this woody vine grows in nitrogen-rich alkaline soils that are both well-drained and moist (Reznicek et al. 2011). *Rubus caesius* prefers to grow in full sun to partial shade but can often adapt to successfully grow within densely shaded areas. Therefore, it is most commonly found in floodplains and riparian zones but can also be found along the forest edges (Reznicek et al. 2011).

*Rubus caesius* was not historically considered a threat to the communities in inhabits in western New York. However, multiple locations have been identified recently, indicating
that *R. caesius* is more prevalent than it was previously thought. It has been observed to grow densely, leading to concerns about restoration and native habitat preservation. Treatment efforts have been applied at Ganondagan State Historic Site in Victor, NY and Green Lakes and Chittenango State Parks in Syracuse, NY. Satellite locations have been found downstream of monoculture areas, leading to concerns about continued spread throughout watersheds. However, it is unknown how *R. caesius* is dispersing. It is also not entirely clear if all identified locations of *Rubus caseius* are correctly identified, as different characteristics in phenetics have been observed throughout the locations in the Finger Lakes Region of New York. Our initial observations indicate that plants in the western locations, including the Brockport and Ganondagan locations exhibited longer petiolules, a longer terminal leaflet, and fewer fruits (as a single drupe) than the Syracuse (Green Lakes field, Green Lakes lot, and Chittenango) locations (Plate 1).

It is important to learn more about these *R. caesius* locations within the Finger Lakes region to determine further best management practices. The first objective of our study is to evaluate the dispersal techniques of *R. caesius* through a greenhouse cutting experiment and quantification of the floristic, and fruiting characteristics among locations. The second objective of our study is to investigate if the purported locations of *Rubus caesius* are identified correctly and morphometrically and genetically similar to one another through vegetative morphometrics and SSR markers. Based on our field observations we hypothesize that the locations in Brockport, Honeoye, and Ganondagan will cluster based on morphometrics and be genetically distinct from locations in Syracuse, NY. We expect that the locations in Green Lakes and Chittenango are genetically different than the other locations of *R. caesius*. We also hypothesize that the locations in Brockport, Honeoye, and
Ganondagan, NY are reproducing mostly vegetatively, while the locations in Syracuse are capable of reproducing both by seed and vegetatively.

**Methods:**

*Site description*

This study was conducted at six *Rubus caesius* locations: Brockport (43.2135, -77.9527), Honeoye (42.8263, -77.5321), Ganondagan (42.9601, -77.4234), and Green Lakes State Park (43.0516, -75.9749, 43.0305, -75.5829) and Chittenango Falls State Park (42.9787, -75.8423), NY (Plate 1). The location in Brockport, NY sits along the west side of a parking lot near a forested park. This location was first discovered in 2020. The Honeoye location is located along Honeoye Creek within the Honeoye Creek Wildlife Management Area. This location was first discovered in 2021. The location in Ganondagan, NY spans along Great Brook Stream within the Ganondagan State Historic Site. This location was discovered in 2018. There are three locations in Syracuse, NY. The first is at Chittenango State Park located along the waterfall trail. This location was first observed in 2019 and is encroaching on sensitive ecological areas. The next two locations are at Green Lakes State Park. The first is on the east side of the park located in a large field. This location was discovered in 2018. The other location is located on the edges of the Meadow Overlook parking lot. This location is the most recent to be discovered in the park in 2021. At each of these locations, the *R. caesius* locations are growing densely.

*Sampling design and environmental measurements*

We established twenty 1m² quadrats each of the five 400m² plots at each location. In each of these quadrats, we measured the soil moisture with a LabQuest Hydrosense 2 probe.
and canopy cover with a spherical crown densiometer. We then selected the lowest canopy cover and most environmentally similar of the plots for comparison among locations (Cornelissen et al. 2003).

Vegetative morphometrics

In each established 1m² quadrat, we selected one *R. caesius* cane to measure vegetative morphometrics in the lab (described below), totaling 20 canes for each location. We cut the individuals at the base, wrapped them in a wet paper towel, and placed them in a cooler with ice to be transported back to the lab.

Once we were back in the lab, we rehydrated the bundles for 20 minutes. We then selected the youngest, two undamaged and fully expanded sun leaves from each cane and cut them at the node and separated them using a razor to ensure no overlap during scanning. We used a CanoScan LiDE 110 scanner to scan the individuals. When we finished scanning, each leaf was put into a coin envelope and dried at 70°C for 48 hours. Once the 48 hours passed, we place the samples in a desiccator for 5 minutes prior to being weighted. We then weighed the samples to the nearest thousandth of a gram. We used Image J software to take morphometric measurements of the scanned images. We measured the area, length, and width of the terminal and lower leaflets, the petiolule length, and the total length and area of the entire three leaflets. We measured the specific leaf area (SLA) by dividing the total leaf area by the dry leaf mass.

Reproductive traits

We counted the number of flowering canes out of 100 randomly selected canes at each of the locations within the plot used for vegetative sampling. If the cane held flowers,
we counted how many flowers there were on the individual cane. In the established quadrats from the vegetative sampling, we counted the number of aggregates per cane, number of drupes per aggregate, and total number of drupes. We collected all aggregates within the ten quadrats at each location. The seeds were removed, air dried on a paper towel overnight, and weighed to the nearest thousandth of a gram to determine the average seed mass per 20m².

**Vegetative propagation**

We conducted a greenhouse experiment to determine if *R. caesius* could vegetatively reproduce. We repeated this experiment 3 times: once in May, again in July, and lastly in September. For this portion of the experiment, we randomly selected 12 *Rubus caesius* individuals and collected the top and bottom 10 cm of each plant from each of the seven locations. We placed these cuttings randomly into standard potting soil in 3in square pots. The soil moisture was monitored to make sure it remained between 20-30 percent soil moisture as observed in the field. The fragments were exposed to natural light with the addition of drop cloths to reduce the amount of heat within the greenhouse. The light availability measured roughly 1500 µmol m⁻² s⁻¹. For eight weeks, the fragments were watered daily and monitored weekly for root development. We marked if each cutting had formed a root. If roots developed, then the fragment was considered a successful propagule.

The greenhouse ranged between 23-30°C. During week six of the first round, an extreme heat event occurred in which the greenhouse reached 39°C. After this extreme heating event, we decided to move the fragments to a location ranging between 22-25°C if the temperature was to exceed 30°C. In the alternate indoor lab, the amount of natural light was lower, with a measure of 900 µmol m⁻² s⁻¹. The fragments were returned to the greenhouse once the temperature reached within range.
**Data analysis**

We averaged the data across the quadrats to determine the average morphometric variable values for the ratio of top leaf length to lower left leaf length, top leaf width to lower left leaf width, top leaf area to lower left leaf area, petiolule length to total leaf length, specific leaf area, number of flowers per 100 canes, flowers per cane, drupes be quadrat, aggregates per cane, drupes per aggregate and seed mass.

To investigate the morphological differences in the vegetative characteristics among the locations, we conducted a series of multivariate analyses. The morphological characters were analyzed using principal component analysis (PCA) and cluster analysis using SPSS. A Kolmogorov-Smirnov test for normality and Levene’s test for homoscedasticity were used to test for assumptions. After all assumptions were satisfied, two one-way ANOVAs were conducted to determine the differences between the environmental characteristics of soil moisture and light conditions among the locations. We conducted a MANOVA and post-hoc Tukey’s test to determine which of the locations were different in terms of vegetative, floral, and fruit characteristics. For the greenhouse experiment, we determined the percentage of individuals that formed roots and were alive for each of the locations for weeks two, four, and eight.

**Genetics**

To determine the genetic differences among and within the different *R. caesius* locations, we utilized four microsatellite primers from published studies (Sarhanova et al. 2017, Graham et al. 2004, Table 1). We collected four random *R. caesius* terminal leaflet samples from each of the six locations totaling 24 samples. We also collected two random *R.*
occidentalis and R. ideaus terminal leaflet samples from each of the six locations to be used as outgroups. We dried these samples using silica gel beads. Terminal leaflet samples were also taken from four herbarium specimens identified as R. caesius from the New York State Herbarium. We used the herbarium specimens to compare current samples of R. caesius to individuals closer to the initial introduction. Once the plant material was dry, we collected 0.5g from each of the samples and ground the material using microbead tubes to prepare for extraction. We extracted the DNA using an AccuPrep DNA extraction kit (BIONEER). We followed protocol according to the package directions. Once the DNA was extracted, we conducted PCR of the microsatellite pairs using a AccuPower PCR PreMix Kit (BIONEER) under the conditions from Sarhanova et al. (2017) as follows: initial activation 95 °C for 15 minutes followed by 38 cycles of 94 °C for 30 seconds, 58-62 °C (depending on loci; Table 1) for 90 seconds, 72 °C for one minute, and a final extension step at 60 °C for 30 minutes (Sarhanova et al. 2017). We purified the PCR products by gel electrophoresis in agarose followed by band isolation with a 400 base pair size standard. With successful amplification by electrophoresis, we sent the samples for fragment analysis to the Biotechnology Resource Center at Cornell University.

Data analysis-genetics

To quantify the genetic differences among the six locations of Rubus caesius, we conducted gel electrophoresis for each primer. The genetic variability was analyzed under the assumption of Hardy-Weinberg equilibrium. The numbers of alleles (Na), numbers of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He) and Nei’s gene diversity index were calculated using GenAIEx 6.41 software (Peakall and Smouse 2006, 2012). The total genetic variation among the samples was calculated using the phi-
statistic through the analysis of molecular variance (AMOVA). This analysis was performed using the GenAlEx 6.41 software. The pairwise Nei’s genetic distances among locations were calculated using the GenAlEx 6.41 software. To determine whether geographic distance calculated with ArcGIS was correlated with genetic distance, a Mantel test was conducted with the GenAlEx 6.41 software. A cluster analysis for the locations was conducted with the Soft Genetics Gene Marker software based on Nei’s genetic distances using the unweighted pair group method with arithmetic mean. We ran a Pearson’s correlation matrix comparing the descriptive statistics and the morphometric variables. Nei’s genetic distance was also used to conduct a principal coordinates analysis (PCoA) using the GenAlEx 6.41 software. We were not able to determine the size of the alleles using the 35a primer, so it was removed from these analyses.

Results:

Selected plots from the six locations ranged from 17 to 23 percent average canopy cover and 27 to 35 percent soil moisture (Figure 1). Neither soil moisture ($F_{(5,114)} = 1.237$, $p=0.297$) nor canopy cover ($F_{(5,114)} = 1.016$, $p=0.412$) differed significantly among the sites.

Vegetative morphometrics

The Green Lakes field location was distinct from the other locations based on a PCA analysis (Figure 3). The Green Lakes field individuals were found on the lower value of the x-axis, which represented a smaller SLA value in the PCA. There were no other distinct separations among locations on the x-axis. Axis 2 represented the ratios of terminal leaf and lower left leaf width, length and area, and the ratio of petiolule length to total leaf length, but there was no visible separation among the locations. There was a significant difference
among the locations in SLA (F(5,114), p=0.00, Figure 4). The Ganondagan, Brockport, and Chittenango locations had the largest SLA and were distinct from the Honeoye and Green Lakes locations. The Green Lakes field locations had the smallest SLA.

The Green Lakes field locations separated from the other locations at the first branch of the cluster analysis (Figure 5). The next separation differentiated the Ganondagan and Brockport locations from the Green Lakes lot, Honeoye, and Chittenango locations.

Reproductive traits

All reproductive traits measured were significantly different among locations (Table 3). The Green Lakes locations had significantly more flowers and drupes per cane than the western locations or Chittenango Falls state park (Figure 6). There was also a significant difference between the two Green Lakes locations. The Green Lakes field location was significantly larger than the Green Lakes lot location for all traits but seed mass, drupes per quadrat, and aggregates per cane. Overall, the Ganondagan and Brockport locations and the Green Lakes location were most similar to one another, while the Chittenango and Honeoye locations varied in similarity to other sites.

Greenhouse

*Rubus caesius* successfully produced roots from cuttings in all locations (Table 3). During the spring, a majority of cuttings produced roots by week four and 75%-95.8% of the canes successfully rooted by week eight. The Green Lakes field and lot locations were most successful in overall survival. A heat event in week 6 killed many of the cuttings for all the locations except for the Ganondagan location. The Ganondagan location was the only one that had an increase in the production of roots after the heat event. In the summer collection,
roots were produced by week 2 and all roots had been produced by week 4 in five of six locations. Root production was lowest during the summer collection with less than half of canes producing roots for all locations except Chittenango. During the fall collection, all locations had formed roots by week four. This round showed the most variability among the locations with a range of 41.4-75% having roots by week eight.

**Genetics**

Amplification was not successful using the 35a, 47a, B06, and G16 primers for *R. occidentalis* and *R. idaeus* samples. The herbarium samples also did not successfully amplify. There was successful amplification of all samples from the six locations of *R. caesius*.

The analysis of genetic diversity among 96 samples of *R. caesius* showed that the average number of alleles, number of effective alleles, observed heterozygosity, and expected heterozygosity were 3.5, 2.931, 0.819, and 0.601, respectively (Table 4). These results do not suggest genetic variation among the locations. In general, the Brockport location showed the most genetic diversity with the highest values of heterozygosity and the number of alleles (Table 4). The Honeoye location was generally the least genetically diverse with the lowest average values of heterozygosity. There were no significant correlations between genetic descriptive statistics and morphometric variables, but some correlations have large correlation coefficients (Table 5). The largest correlation coefficients were found between the ratio of top leaf width to lower left leaf width and gene distance and Ne as well as seed mass to observed heterozygosity (r>0.7, Table 5).
The results of AMOVA suggest significant minor genetic differences among locations. Specifically, 20% of the total genetic variance (PhiRT) was found among locations. The next level, among individuals within locations contributed 66% of the total genetic variance (PhiPR), and the remaining 14% genetic variance was obtained from within individuals (PhiPT). All of these contributed significantly to the overall genetic variation (p=0.001, Table 6).

Separate cluster analyses for each of the four primers based on Nei’s genetic distance showed evidence for variation among locations in the 47a and G16 primers (Figure 7). For the 47a cluster analysis, the Brockport, Ganondagan, Honeoye and Green Lakes Lot grouped together in the first cluster. The second cluster consisted of the Green Lakes Field and Chittenango locations. For the G16 primer, the first cluster contained the Brockport, Chittenango, and Ganondagan locations. The second cluster contained the Honeoye, Brockport, and Chittenango locations. The third cluster contained the Honeoye, Ganondagan, Green Lakes Lot and Green Lakes field locations. The six locations did not show distinct separation through cluster analysis based on Nei’s genetic distance for all four primers combined (Figure 8).

Based on Principal Coordinate Analysis, the Green Lakes Field and Ganondagan locations were distinct from other locations based on genetic distance. The Honeoye and Brockport locations were grouped together as well as the Chittenango and Green Lakes Lot locations (Figure 9). The Mantel test showed no significant correlation between genetic distance and geographical distance among the locations (R^2=0.0006, Figure 10).
Discussion:

*Rubus caesius* is a serious and increasing threat to native habitats in western New York. Over the last five years multiple locations of *R. caesius* have been identified across the region. It has been observed to form monocultures and spread down riparian zones. With increased distribution of *R. caesius*, we set out to determine the identity, morphology, and dispersal traits of purported *R. caesius* across western New York. This information is crucial for invasive species management because successful eradication and control depends on species identity and species function.

We confirmed that the six locations studied were correctly identified as *Rubus caesius*. We used four microsatellite primers provided by Sarhanova et al. (2017) that were known to amplify *R. caesius*. All samples from the purported *R. caesius* locations amplified, while the outgroup samples of *R. occidentalis* and *R. idaeus* did not. This makes sense because microsatellite primers are designed to be species-specific and investigate specific segments of the species’ genome. Herbarium specimen samples obtained from the New York State Museum Herbarium also did not amplify, most likely due to the age of the specimens. Although the *Rubus* genus does frequently hybridize, that was not the case for these locations of *R. caesius*. Sarhanova et al. (2017) found that their data pointed to hybridization between apomicts as pollen donors and sexuals as mother plants. Sochor et al. (2015) supports this with the study of *Rubus radula* which was derived from the cross ser. *Discolores* x ser. *Glandulosi*. To look deeper into possible hybridization within the location it would be beneficial to utilize more primers in order to get a bigger picture of the differences within the genome. With the use of more primers, there is a greater chance of determining small differences in the repeated sections and among the locations.
As expected, we found among-location differences based on vegetative traits, reproductive traits, and genetic analyses. Interestingly, differences among locations did not solely reflect geographic distance, contrary to our hypothesis. Two of the western locations, Ganondagan and Brockport, were most similar to each other when comparing vegetative and reproductive traits, but the third western locations, Honeoye, often clustered with the Green Lakes location. It is possible that the Honeoye location could be a result of an introduced propagule from Green Lakes since there was such similarity between them, or vice versa. They may also have come from the same initial stock of Rubus fragments used to control erosion by the NYS DOT (Kyle Webster, personal communication). Similarly, the Chittenango location, in the east, was more similar to the Ganondagan and Brockport locations. Our Mantel test confirmed the lack of relationships between geographic distance and genetic differences.

Existing trait and genetic variation among locations could be due to local adaptations in addition to different source fragments or seeds. The rapid evolution of local adaptation may enable invasive plant species to thrive across a broad range of habitats (Ellstrand and Schierenbeck 2000, Reznick and Ghalambor 2001). A study investigating the local adaptation tendencies of the invasive Ambrosia artemisiifolia found that local adaptation is a significant driver for invasive success (Boheemen et al. 2018). However, our genetic analyses only indicate weak genetic differentiation among sites. Local adaptation has not resulted in large genetic differences up to this point. These locations are most likely reproductively isolated given the low flower production and large geographic distances, with the exception of the Green Lakes locations. There is the potential for increased genetic differentiations among these locations in the future.
Specific leaf area differentiated sampled locations, and this could reflect environmental or genetic differences. SLA was smallest in the Green Lakes sites and at Honeyoe. Differences in SLA could reflect genetic differences or could be due to phenotypic plasticity: SLA changes with light, nutrients, and moisture differences (Ackerly et al. 2002, Celis et al. 2017). Lower SLA is associated with increased light and/or lower moisture availability (Burns 2004, Wright and Westoby 1999). The sites that did have the lowest SLA did not have lower soil moisture, and there was no difference in canopy cover. Previous research has shown that SLA varies more based on climate variables such as MAT and MAP than other environmental variables (Liu et al. 2017). *Rubus caesius* may be adapting different SLA due to characteristics of different environments. For example, even though we collected samples from well-lit areas to account for environmental plasticity, most of the Ganondagan location is shaded and along a riparian zone, so directional selection in SLA could be occurring across the site to increase average specific leaf area. Scheepens et al. (2010) conducted a common garden experiment that showed differently located populations of *Campanula thyrsoides* showed a decreased SLA as a possible adaptation to drought conditions. A common garden experiment is necessary to fully investigate leaf traits for plasticity vs. genetic differences (e. g. Geng et al. 2006, Scheepens et al. 2010).

Interestingly, we determined that there was variation in the reproductive traits of *R. caesius*. These locations of *R. caesius* could have different life history strategies due to founder effects or rapid evolution due to abiotic and/or biotic factors (Muller-Scharer et al. 2004). The Green Lakes field and lot locations showed significantly greater amounts of flowering and seed production, but lower seed mass. The rest of the locations produced very few flowers but had a greater seed mass. Studies have shown that seed output is negatively
correlated with seed mass (Westoby et al. 2002). Species with larger seed masses establish more successfully under hazardous conditions such as more shade and lower soil moisture (Westoby et al. 2002). Smaller seeds generally germinate faster, but larger seeds germinate slower and with higher success (Castro 1999, Suarez-Vidal et al. 2016). Reproductive traits were most highly correlated with our genetic analyses, although not significantly, so future research should increase samples sizes to further investigate these correlations.

*Rubus* species are known to possess tradeoffs between reproductive and vegetative growth to improve reproductive strategies (Nagarkar 2012, Suzuki 1987). For example, *R. odoratus* has a tradeoff between fruit production and vegetative growth because these growth processes occur at the same time (Nagarkar 2012). This, however, can depend on numerous outside environmental factors (Pers-Kamczyc 2022, Muller-Scharer et al. 2004). In work on a related species that is also invasive, Amsellem et al. (2001) used microsatellite analysis to investigate the possible shifts of the reproductive biology of *R. alceifolius* in the introduced and native range. They found that the differences in reproductive strategies of *Rubus alceifolius* was largely due to sexual reproduction. We know that *R. caesius* is capable of reproducing both sexually and asexually (Lewin 2016), so it is possible that *R. caesius* locations have shifted reproductive strategies like its relative *R. alceifolius*.

Throughout western New York *R. caesius* has the potential to reproduce by vegetative fragments. Based on evidence of downstream propagation, this confirmed our hypothesis. Riparian zones are susceptible to continued disturbance due to fluctuating water levels (Catford and Jansson 2014, Aronson et al. 2017). This makes management potentially difficult because the fragments can easily spread throughout the water systems through hydrochory (Nilsson et al. 2010). Hydrochory has been inferred to be an important vector for
the spread of many invasive species (Lonsdale 1993, Schneider and Sharitz 1988). This allows plants to colonize sites out of reach with other dispersal vectors. For example, the actual rates of spread of the invasive *Mimosa pigra* were four times faster via hydrochory compared to being dispersed by other means (Lonsdale 1993). Propagules are able to travel much longer distances and are likely to germinate and establish due to the continually disturbed landscape (Schneider and Sharitz 1988). Other invasive *Rubus* species, such as *R. phoenicolasius*, also spread throughout watersheds through fragmentation (Foss 2005). This species is managed through fragment-reducing techniques such as chemical treatments (Foss 2005).

Our results demonstrate that management strategies may need to differ based on the location under focus due to the differences in vegetative and reproductive traits among locations. With the locations of *R. caesius* that produce significant amounts of seed and reproduce via fragmentation, it would be crucial to apply treatment before there is an opportunity to produce seed. The timing of treatment may not be as important in low-fruiting locations. Overall, the treatment strategies chosen should decrease the number of fragments created either by bagging the material after treatment, or by not using fragment-inducing treatment at all. Future research on treatments is important to determine which management methods are best for eradication.
Literature Cited:


Li, X., Y. Li, and X. Li. 2015. Influences of environmental factors of leaf morphology of Chinese jujubes. PLOS ONE 10(5): e0127825.


PRIMER-e. 2017. PRIMER 7. PRIMER-e.


**Tables:**

**Table 1.** Microsatellite primers for *R. caesius* and PCR conditions for each (Sarhanova et al. 2017).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer</th>
<th>Annealing temperature</th>
<th>Florescent dye</th>
<th>Primers concentration [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubusr35a</td>
<td>F: ttggaaggcacaagcagata</td>
<td>58 °C</td>
<td>5’6-FAM</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>R: ggcacacagcacaacaaagt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rubusr47a</td>
<td>F: aagcaggacacctcagatge</td>
<td>62 °C</td>
<td>5’6-FAM</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>R: cagccaaacattcagcta</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERubLF_SQ 01_B06</td>
<td>F: cctctacacccctcagctc</td>
<td>60 °C</td>
<td>5’6-FAM</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>R: cgtagccagctctctctgt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERubLF_SQ 01_G16</td>
<td>F: gcaccctaattctcatgtc</td>
<td>60 °C</td>
<td>5’6-FAM</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>R: atctcgtttcetctcacc</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Percentage of *R. caesius* individuals rooting and survival every two weeks from six locations during three greenhouse experiments in the spring, summer, and fall of 2021.

<table>
<thead>
<tr>
<th>Season</th>
<th>Week</th>
<th>Brockport % Root, Survival</th>
<th>Honeoye % Root, Survival</th>
<th>Ganondagan % Root, Survival</th>
<th>Green Lakes Field % Root, Survival</th>
<th>Green Lakes Lot % Root, Survival</th>
<th>Chittenango % Root, Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0, 100</td>
<td>0, 100</td>
<td>0, 100</td>
<td>0, 100</td>
<td>0, 100</td>
<td>0, 100</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>75, 33</td>
<td>75, 66.7</td>
<td>70.8, 41.7</td>
<td>70.8, 50</td>
<td>75, 50</td>
<td>66.7, 41.7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>91.7, 58.3</td>
<td>83.3, 62.5</td>
<td>70.8, 45.8</td>
<td>95.8, 37.5</td>
<td>91.7, 58.3</td>
<td>79.2, 54.2</td>
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<tr>
<td></td>
<td>6</td>
<td>91.7, 8.3</td>
<td>83.3, 16.7</td>
<td>75, 45.8</td>
<td>95.8, 20.8</td>
<td>95.8, 20.8</td>
<td>79.2, 12.5</td>
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<td>8</td>
<td>41.7, 33.3</td>
<td>37.5, 20.8</td>
<td>45.8, 20.8</td>
<td>45.8, 70.8</td>
<td>41.7, 33.3</td>
<td>54.2, 41.7</td>
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<td>4</td>
<td>41.7, 8.3</td>
<td>37.5, 4.2</td>
<td>45.8, 8.3</td>
<td>45.8, 41.7</td>
<td>41.7, 41.7</td>
<td>54.2, 4.2</td>
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<td>6</td>
<td>45.8, 8.3</td>
<td>37.5, 4.2</td>
<td>45.8, 8.3</td>
<td>45.8, 25</td>
<td>41.7, 25</td>
<td>54.2, 4.2</td>
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<td></td>
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<td>45.8, 8.3</td>
<td>37.5, 4.2</td>
<td>45.8, 8.3</td>
<td>45.8, 25</td>
<td>41.7, 25</td>
<td>54.2, 4.2</td>
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<td>0, 100</td>
<td>0, 100</td>
<td>0, 100</td>
<td>0, 100</td>
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<td>70.8, 58.3</td>
<td>54.2, 75</td>
<td>54.2, 70.8</td>
<td>41.7, 33.3</td>
<td>58.3, 66.7</td>
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<td>6</td>
<td>54.2, 33.3</td>
<td>70.8, 45.8</td>
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<td>66.7, 54.2</td>
<td>41.7, 41.7</td>
<td>62.5, 37.5</td>
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<td></td>
<td>8</td>
<td>58.3, 33.3</td>
<td>75, 45.8</td>
<td>62.5, 58.3</td>
<td>70.8, 54.2</td>
<td>26, 25</td>
<td>70.8, 33.3</td>
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</tbody>
</table>
Table 3. Results of one-way MANOVA on reproductive traits of *Rubus caesius* from six locations in western New York. N=20 samples from each location.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>F</th>
<th>Df1</th>
<th>Df2</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of flowering canes per 100 canes per quadrat</td>
<td>88.12</td>
<td>5</td>
<td>114</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of flowers per cane</td>
<td>30.664</td>
<td>5</td>
<td>114</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of drupes per quadrat</td>
<td>93.604</td>
<td>5</td>
<td>114</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of aggregates per cane</td>
<td>4.801</td>
<td>5</td>
<td>114</td>
<td>&lt;0.0001</td>
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<tr>
<td>Seed Mass (g)</td>
<td>6.268</td>
<td>5</td>
<td>114</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of aggregates per cane</td>
<td>4.801</td>
<td>5</td>
<td>114</td>
<td>&lt;0.0001</td>
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</table>
Table 4. Descriptive statistics of number of alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), and the F-statistic (F) for the 47a, B06, and G16 SSR primers.

<table>
<thead>
<tr>
<th>Location</th>
<th>Locus</th>
<th>Sample Size</th>
<th>Na</th>
<th>Ne</th>
<th>Ho</th>
<th>He</th>
<th>F</th>
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<tbody>
<tr>
<td>Brock</td>
<td>47a</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>0.5</td>
<td>0.75</td>
<td>0.333</td>
</tr>
<tr>
<td></td>
<td>B06</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>G16</td>
<td>4</td>
<td>7</td>
<td>6.4</td>
<td>0.75</td>
<td>0.844</td>
<td>0.111</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>4.667</td>
<td>4.133</td>
<td>0.750</td>
<td>0.698</td>
<td>0.481</td>
</tr>
<tr>
<td>Hon</td>
<td>47a</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B06</td>
<td>4</td>
<td>3</td>
<td>2.462</td>
<td>1</td>
<td>0.594</td>
<td>-0.684</td>
</tr>
<tr>
<td></td>
<td>G16</td>
<td>4</td>
<td>3</td>
<td>2.462</td>
<td>0.5</td>
<td>0.594</td>
<td>0.158</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>2.000</td>
<td>1.641</td>
<td>0.500</td>
<td>0.396</td>
<td>-0.175</td>
</tr>
<tr>
<td>Gan</td>
<td>47a</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0.5</td>
<td>-1</td>
</tr>
<tr>
<td></td>
<td>B06</td>
<td>4</td>
<td>4</td>
<td>3.556</td>
<td>1</td>
<td>0.719</td>
<td>-0.391</td>
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<tr>
<td></td>
<td>G16</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>0.75</td>
<td>-0.333</td>
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<tr>
<td>Average</td>
<td></td>
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<td>3.333</td>
<td>3.185</td>
<td>1.000</td>
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<td>GLF</td>
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<td>0.625</td>
<td>-0.6</td>
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<td>G16</td>
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<td>4</td>
<td>3.2</td>
<td>1</td>
<td>0.688</td>
<td>-0.455</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>3.667</td>
<td>3.022</td>
<td>1.000</td>
<td>0.667</td>
<td>-0.503</td>
</tr>
<tr>
<td>GLL</td>
<td>47a</td>
<td>4</td>
<td>2</td>
<td>1.28</td>
<td>0.25</td>
<td>0.219</td>
<td>-0.143</td>
</tr>
<tr>
<td></td>
<td>B06</td>
<td>4</td>
<td>3</td>
<td>2.667</td>
<td>1</td>
<td>0.625</td>
<td>-0.6</td>
</tr>
<tr>
<td></td>
<td>G16</td>
<td>4</td>
<td>5</td>
<td>3.2</td>
<td>0.75</td>
<td>0.688</td>
<td>-0.091</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>3.333</td>
<td>2.382</td>
<td>0.667</td>
<td>0.511</td>
<td>-0.278</td>
</tr>
<tr>
<td>Chitt</td>
<td>47a</td>
<td>4</td>
<td>4</td>
<td>3.2</td>
<td>1</td>
<td>0.688</td>
<td>-0.455</td>
</tr>
<tr>
<td></td>
<td>B06</td>
<td>4</td>
<td>3</td>
<td>2.462</td>
<td>1</td>
<td>0.594</td>
<td>-0.684</td>
</tr>
<tr>
<td></td>
<td>G16</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>0.75</td>
<td>-0.333</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>4.000</td>
<td>3.221</td>
<td>1.000</td>
<td>0.677</td>
<td>-0.491</td>
</tr>
<tr>
<td>All Loci-Average</td>
<td></td>
<td></td>
<td>3.500</td>
<td>2.931</td>
<td>0.819</td>
<td>0.601</td>
<td>-0.257</td>
</tr>
</tbody>
</table>
Table 5. Pearson’s correlation coefficients among morphometric and genetic variables from six locations. Variables include: ratio of top leaf length to lower left leaf length (TL Length/LL Length), top leaf width to lower left leaf width (TL Width/LL Width), top leaf area to lower left leaf area (TL Area/LL Area), petiolule length to total leaf length (PL/To. Length), specific leaf area (SLA), number of flowers per 100 canes, flowers per cane, drupes be quadrat, aggregates per cane, drupes per aggregate and seed mass to genetic variables of number of alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), and genetic distance.

<table>
<thead>
<tr>
<th>Morphometric Variables</th>
<th>Na</th>
<th>Ne</th>
<th>Ho</th>
<th>He</th>
<th>Nei’s Genetic Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL Length/LL Length</td>
<td>0.314</td>
<td>0.622</td>
<td>0.378</td>
<td>0.61</td>
<td>-0.329</td>
</tr>
<tr>
<td>TL Width/LL Width</td>
<td>-0.539</td>
<td>-0.94</td>
<td>0.471</td>
<td>0.124</td>
<td>-0.719</td>
</tr>
<tr>
<td>TL Area/LL Area</td>
<td>-0.073</td>
<td>0.159</td>
<td>0.389</td>
<td>0.13</td>
<td>-0.174</td>
</tr>
<tr>
<td>PL/To. Length</td>
<td>0.416</td>
<td>0.336</td>
<td>0.489</td>
<td>0.21</td>
<td>-0.506</td>
</tr>
<tr>
<td>SLA</td>
<td>0.56</td>
<td>0.566</td>
<td>-0.16</td>
<td>0.378</td>
<td>0.31</td>
</tr>
<tr>
<td># Flowers/100 canes</td>
<td>-0.195</td>
<td>-0.186</td>
<td>0.206</td>
<td>-0.044</td>
<td>-0.167</td>
</tr>
<tr>
<td>Flowers per cane</td>
<td>0.058</td>
<td>-0.259</td>
<td>-0.091</td>
<td>-0.303</td>
<td>0.248</td>
</tr>
<tr>
<td>Drupes per quadrat</td>
<td>-0.177</td>
<td>-0.193</td>
<td>0.538</td>
<td>-0.085</td>
<td>-0.428</td>
</tr>
<tr>
<td>Aggregates per cane</td>
<td>-0.191</td>
<td>-0.239</td>
<td>0.381</td>
<td>-0.126</td>
<td>-0.319</td>
</tr>
<tr>
<td>Drupes per aggregate</td>
<td>-0.257</td>
<td>-0.168</td>
<td>0.364</td>
<td>0.026</td>
<td>-0.464</td>
</tr>
<tr>
<td>Seed mass (g)</td>
<td>-0.239</td>
<td>-0.258</td>
<td>0.706</td>
<td>-0.163</td>
<td>-0.592</td>
</tr>
</tbody>
</table>
**Table 6.** AMOVA of the gene distance matrix using four primers shows the proportion of variation attributable to each source.

<table>
<thead>
<tr>
<th>Source of Variance</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>Est. Variance</th>
<th>%</th>
<th>Phi Statistic</th>
<th>Value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among locations</td>
<td>5</td>
<td>6.813</td>
<td>1.363</td>
<td>0.090</td>
<td>20%</td>
<td>PhiRT</td>
<td>0.202</td>
<td>0.001</td>
</tr>
<tr>
<td>Among individuals within</td>
<td>18</td>
<td>11.625</td>
<td>0.646</td>
<td>0.292</td>
<td>66%</td>
<td>PhiPR</td>
<td>0.824</td>
<td>0.001</td>
</tr>
<tr>
<td>locations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among individuals</td>
<td>24</td>
<td>1.500</td>
<td>0.063</td>
<td>0.063</td>
<td>14%</td>
<td>PhiPT</td>
<td>0.859</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Plates:

Plate 1. Two morphotypes present in the Finger Lakes region of New York. The left shows an individual from Green Lakes State Park in Syracuse, NY. The right shows an individual from Ganondagan State Historic Site in Ganondagan, NY.
Figures:

**Figure 1.** Average soil moisture (a) and percent canopy cover (b) at the six locations of *Rubus caeius*. Error bars represent standard error.
Figure 2. Map showing the six locations of *R. caesius* across the Finger Lakes region of New York. The western locations include Brockport, Honeoye, and Ganondagan. The Eastern locations include Green Lakes field, Green Lakes lot, and Chittenango.
Figure 3. Principal components analysis (PCA) of *Rubus caesius* individuals from six locations (symbols) based on vegetative morphometric analyses. The traits include terminal leaflet width to lower left leaflet width (TL W/LL W), terminal leaflet area to lower left leaflet area (TL A/LL A), terminal leaflet length to lower left leaflet length (TL L/LL L), petiolule length to total leaflet length (PL/TO L), and specific leaf area (SLA).
Figure 4. Specific leaf area (g/cm²) of six locations of *R. caesius*. The error bars represent standard error. Sites that do not share the same letter were significantly different from each other based on a Tukey’s test after a one-way ANOVA.
Figure 5. Cluster analysis based on the Bray Curtis similarity of *R. caesius* individuals in six locations (symbols) based on vegetative morphometric analyses.
Figure 6. Reproductive traits of Rubus caseius from six locations in western New York. Different letters indicate differences among locations after a MANOVA and post-hoc Tukey’s test.
Figure 7. Dendrogram of the six locations of *R. caesius* based on Nei’s genetic distance for each of the microsatellite primers.
Figure 8. Dendrograms of the six locations of R. caesius based on Nei’s genetic distance using the 35a, 47a, B06, and G16 microsatellite primers.
**Figure 9.** Principal Coordinate Analysis of the gene distance of 24 *R. caesius* individuals from six locations: Ganondagan (GAN), Honeoye (HON), Brockport (BROCK), Green Lakes Field (GLF), Green Lakes Lot (GLL), and Chittenango (CHITT).
Figure 10. Mantel Test on between Nei’s genetic distance and the longitudinal distance among locations. Each point represents an individual (N=24). X-axis represents the Nei’s genetic distances among the locations, and the Y-axis represents the geographical parameter ($R^2=0.0006$).
Chapter 2

Chemical treatment is more effective than mechanical control for reducing invasive *Rubus caesius* cover in riparian systems.

**Introduction:**

The colonization of invasive species is rapidly increasing due to human travel, trade, and disturbance (Mack *et al.* 2000, Vitousek *et al.* 1997). Once present in a community, invasive species can disrupt plant communities and cause long-term impacts on ecosystem processes by outcompeting native species for the key resources (Kuebbing *et al.* 2014, Mack *et al.* 2000). The characteristics and effects of these non-native invaders pose a threat to the biodiversity of their invaded ecosystem, often to the point of extirpation of native species (Anderson *et al.* 1996). Therefore, it is critical to slow current invasions, and prevent further invasion through conservation efforts (Mack *et al.* 2000, Trebitz and Taylor 2007).

An area of focus in invasive species control efforts is riparian zones. The management and control of riparian invasive species is critical in reducing further colonization (NYDEC 2020). Recurring flood events along the interconnected waterways of riparian zones selects for invasive species through increased competition (Bottollier-Curtet *et al.* 2013). Riparian invasive plants have a higher productivity, a competitive advantage, and a higher resilience toward disturbance at early developmental stages, which results in further invasion success (Bottollier-Curtet *et al.* 2013). The management and control of invasive species is crucial to the future of native landscapes (Mack *et al.* 2000, Trebitz and Taylor 2007).

Invasive plant control is a challenge for natural resource management. Much of the research that has been conducted mainly quantifies the scale of the invasion rather than
finding adaptive solutions (Hume 2006). Therefore, managers often refer to general conclusions of invasive plant management that do not evaluate long-term success of treatment effects (Petersen and Clary 2005). Epanchin-Niell and Hastings (2010) reported that applications of uncertain control efforts result in over-allocated management efforts and insufficient invader control. Determining effective control methods in riparian habitats is critical due to their ecological importance (Aslan et al. 2012). These management decisions about which control method to use depend heavily on the invasive plant species’ growth forms, the economic situation of the inhabited area, and available resources (Weidlich et al. 2020). For these reasons, some of the most common methods that are used are mechanical and chemical, or herbicide, treatments.

Mechanical controls are often used in riparian area control efforts. Mechanical control includes measures of removal such as mowing or hand-pulling. Mechanical methods are often the first utilized because they do not require any licensing or introduce any chemicals into the ecosystem (Richardson et al. 2000). However, mechanical controls are often insufficient and sometimes even promote further invasion by not affecting the whole plant (McHugh 2006). *Reynoutria japonica* (Japanese knotweed) is a common invasive that has significant impacts on riparian ecosystems. Delbart et al. (2012) investigated various mechanical treatments on *R. japonica*. They determined that mechanical treatments were generally less effective than chemical treatments. This, however, is not always the case for all invasive plants. Ingham (2014) determined that mechanical removal by mowing of the invasive *Rubus armeniacus* (Himalaya blackberry) was effective in reducing the percent cover. However, regeneration showed a significant increase in nonnative forbs (Ingham 2014).
Chemical controls are the use of pesticides. These methods are often used in invasive species management because they target plant structures (Delbart et al. 2012). The use of herbicides often shows results in the short term, and when reapplied can have long-lasting results (NY DEC 2020, Delbart et al. 2012). Combined treatments, which are the use of both mechanical and chemical treatments, are often utilized in invasive plant management because this often increases the efficacy of treatment (Mozdzer et al. 2008). Bashtanova et al. (2009) conducted combined mechanical and herbicide treatments to eradicate *Phragmites australis* in the riparian habitat of the Platte River. They determined that mowing followed by herbicide application using Rodeo provided season-long control for up to three growing seasons. The mechanical-only treatment was observed to reduce the initial coverage of *P. australis* but did not last throughout the season. Although chemical control methods are generally the most effective, they cannot always be used by land managers due to legal constraints, possible harmful effects to the environment, and funding constraints (Bashtanova et al. 2009). Riparian zones are common areas for herbicide restrictions due to potential impacts to aquatic systems.

The *Rubus* genus commonly thrives in riparian habitats due to the increased disturbance and dispersal by flood events (Waugh et al. 1990, McDowell and Radosevich 2005). *Rubus caesius* is a riparian invasive woody shrub that is native to Europe and Asia. It has biennial canes and can reproduce both vegetatively via rhizomes and fragmentation (see Chapter 1) and via fruit that is dispersed by birds and mammals (Fernald 1950, Reznicek et al. 2011). In its natural habitat, this woody vine grows in nitrogen rich alkaline soils that are both well-drained and moist (Reznicek et al. 2011). *Rubus caesius* prefers to grow in full sun to partial shade but can often adapt to successfully grow within densely shaded areas. It
commonly invades floodplains and riparian zones outside of its native range and can also be found along the forest edges (Reznicek et al. 2011). In western New York, *Rubus caesius* has been observed to grow densely, often forming monocultures, and spreads downstream throughout watersheds. It has encroached upon the habitat of rare and endangered species, such as *Novisuccinea chittenangoensis* (Chittenango Ovate Amber Snail) at Chittenango Falls State Park and hinders the restoration of native communities at Ganondagan State Historic Site (NYDEC 2022, Friends of Ganondagan 2022).

Effectiveness of control methods for eradicating *R. caesius* are unknown. Therefore, our objective is to assess how to control *R. caesius* by determining the most effective control methods in reducing the cover of *Rubus caesius*. We hypothesize that the most effective treatment will be the combined treatment of cutting and using herbicide, as simply cutting as has not been successful long-term in related species (Renteria et al. 2012).

**Methods:**

This study was conducted at Ganondagan State Historic Site (42.9601, -77.4234) located in the northwestern portion of Ontario County, in the town of Victor, New York. *Rubus caesius* grows densely in the riparian areas of Great Brook stream extending roughly a mile. This site was first identified in the spring of 2018 by NYS Parks staff (Kyle Webster, personal communication). We measured soil moisture using a Hydrosense 2 soil moisture probe and canopy cover using a spherical crown densiometer to find environmentally similar areas for our control experiment. We found *R. caesius* growing in canopy cover greater than fifty percent (range 50-95%), referred to hereafter as low light conditions. Areas of invasion additionally contained *R. caesius* growing in canopy cover lower than fifty percent (range 5-35%), hereafter called high light conditions. We selected five sites that were most
environmentally similar in low-light conditions and two sites that were most environmentally similar in high-light conditions to be used for comparison.

We separated each of the seven treatment areas into plots. Treatments were assigned following a randomized blocked design. Each plot measured roughly 156 square meters in size with the inner 100 square meters being used for sampling. This considered the rhizomatous trait of *R. caesius*, reducing cross contamination of treatments in the sampling areas. We assigned the treatments to each plot using a random number generator. Due to size restrictions three treatments could be applied to most sites. Mechanical removal, mechanical removal followed by herbicide (Rodeo) spraying, and no treatment (control) were chosen as the main treatments (Table 1). At additional sites, we included the treatments of mechanical removal followed by herbicide (Garlon 4 Ultra) spraying, herbicide (Rodeo) treatment only, and mechanical removal repeated twice. Some sites had replicate plots within a treatment (Table 1).

Before treatment was administered, we conducted pre-treatment sampling. In each of the treatment plots, we established ten randomly spaced quadrats within the 100 square meter sampling area. The center of each quadrat was permanently marked to ensure sampling would be conducted in the same location throughout the study. At the center of each quadrat, the percent cover of *Rubus caesius* was noted. Resampling was conducted one month after the herbicide treatment was applied.

*Mechanical treatments*

Members of the Ganondagan Environmental Field Team and I conducted the mechanical removal treatment using a Stihl 111R brush hog with a metal grass cutting blade on August 10 - 11, 2020 and July 25 - 26, 2021. This metal grass cutting blade was chosen
Because we hoped it would produce smaller fragments than a typical line cutter, and we hoped this would reduce the chance of the *R. caesius* sprouting from the cuttings. In each of the mechanical treatment plots, the *R. caesius* canes were cut at the base to the ground throughout the entire plot. For treatment plots receiving the mechanical treatment twice, a cut was administered and the *R. caesius* was given eight weeks to grow back before being cut again by the same means.

*Herbicide treatments*

The mechanical and herbicide treatments using Rodeo were conducted using standard concentrations used by the field team at Ganondagan State Historic Site (1.9 oz/gal concentration). At the additional site with an additional mechanical and herbicide treatment using Garlon 4 Ultra we used standard concentrations used by the field team at Ganondagan State Historic Site (1 oz/gal). An Li700 surfactant was also added to each of the herbicides. On August 10-11, 2020 and July 25-26, 2021 each of the treatment plots receiving the paired treatment were cut with the Stihl brush hog using the grass cutting blade. The *R. caesius* canes were cut to the ground throughout the entire treatment plot. The plots were then treated with the respective herbicide treatments on October 8, 2020 and October 5, 2021.

Plots that received only the herbicide (Rodeo) treatment were treated at the same time as the first herbicide treatment of the mechanical and herbicide paired treatments. The herbicide was applied in the same manner and same concentration. The only difference in this treatment was that the vegetation was not cut back before spraying.

*Statistical Analyses*
Effects of treatments on percent cover of \textit{R. caesius} were evaluated using non-parametric tests, because even after transformation a Kolmogorov-Smirnov test for normality and Levene’s test for homoscedasticity indicated that the data did not satisfy assumptions of ANOVA. Kruskal-Wallis tests were used to evaluate the effect of the three treatments (mechanical, mechanical herbicide (Rodeo), and control (no treatment) on \textit{Rubus caesius} change in cover compared to initial cover in 2020. Percent cover data were averaged across the ten sample quads in each plot before analysis. For sites with replicate plots, one of the two plots was chosen randomly for inclusion in statistical analyses. As there were only two high-light sites, we were not able to statistically evaluate treatment effects in high-light conditions. For each of the two sites that had additional treatments, Kruskal-Wallis tests were used to compare all the treatments with sample quadrats as replicates. Tests were conducted for each of the three treatment years. We used Dunn’s All Pairs for Joint Ranks post-hoc test using a Bonferroni adjustment to look for within year pairwise differences.

\textbf{Results:}

Before treatments were initiated, the initial percent cover of \textit{R. caesius} in the low-light plots was significantly lower in control plots than in plots assigned to the mechanical or combined mechanical and herbicide treatments (Figure 1, Table 2). There was no significant difference between the initial percent cover of \textit{R. caesius} at sites D and E where additional treatments of mechanical removal twice, chemical only using Rodeo, and the combination of mechanical and herbicide using Garlon 4 Ultra were also applied.

Treatments significantly affected \textit{Rubus caseius} cover in both the first and second years of treatments (Table 2). Of the three treatments applied at all sites, the combination treatment of mechanical removal and Rodeo treatments was effective in both years of
treatment in the low and high light conditions, although we could only test statistical significance for the former (Figure 1 and Figure 2). The combination Rodeo-mechanical treatment was also a significantly effective treatment in sustaining a reduction in percent coverage into the next year of treatment compared to the mechanical-only treatment. The mechanical-only treatment was not effective in reducing the percent coverage of *R. caesius* at all the sites in the first or second year of treatment at all of the sites (Figure 1).

For the treatments that were only conducted on a site-specific basis at Site D and E, the herbicide-only application of the Rodeo herbicide significantly reduced coverage of *R. caesius* compared to the mechanical treatments (Figure 3 and Figure 4). The treatment of mechanical removal twice was not successful in reducing the percent coverage of *R. caesius* in the first year of treatment. However, in the second year of treatments, applying mechanical treatments twice significantly reduced *Rubus* cover at both site D and E (Figure 3 and Figure 4, Table 3 and Table 4). The combination treatment of mechanical removal and the Garlon 4 Ultra herbicide application applied only at Site E was successful in eliminating the coverage of *R. caesius* after the first year of treatment compared to the mechanical-only treatment.

**Discussion:**

*Rubus caesius* is a serious threat to the riparian habitats it invades. These riparian habitats are key to ecosystems but are sensitive to invasive species due to continual disturbance by fluctuating water levels. Repeated flooding events results in further propagation down the watershed without proper management techniques. Since *R. caesius* has been observed to have a negative effect on the ecosystem and spread down the watershed, it is important to determine best management practices. Through this study, we
wanted to determine if *R. caesius* could be controlled mechanically, chemically, or with a combination of mechanical and chemical treatments.

Overall, as we expected, applying a mechanical treatment once each year was not successful in reducing the cover of *R. caesius*. The first year of treatment was effective in reducing the amount of cover, but the following year showed an increase surpassing the original coverage amount. Rapp et al. (2012) found similar results in their control experiment of *Phragmites australis*. They determined that the mechanical treatment was not effective in reducing the percent cover of the invasive throughout the season. Delbart et al. (2012) found this to also be the case in the mechanical control of *Reynoutria japonica*. Due to the rhizomatous trait of *R. japonica*, it was difficult to eradicate via mechanical means because the plants could easily continue growth and even spread further after mechanical treatments. Since *R. caesius* is also rhizomatous, mechanical treatments are likely not effective because they leave rhizomes intact. However, the increase in percent cover after the second treatment season could also be due to fragmentation, as *Rubus* species can successfully grow from fragments (Foss 2005).

The repeated mechanical treatment was effective in reducing the coverage of *R. caesius*. The first year of this treatment was not as effective as the second year of treatment. This treatment, however, was only applied at two of the seven sites. DiTommaso et al. (2013) investigated the use of repeated mechanical on the invasive species *Vincetoxicum rossicum*. They determined that the repeated mechanical treatment was effective in reducing the coverage of *V. rossicum*, but it was not as effective as the use of Rodeo and Garlon 4 Ultra treatments (DiTommaso et al. 2013). In this study, although the repeated mechanical treatment was not as effective as chemical use in reducing the coverage, it still serves as an
effective option for *R. caesius* control. A focus group study of forest owners conducted by Howle et al. (2017) found that landowners are commonly concerned about the impacts of the use of chemicals to eradicate invasive species, so further studies including more sites of repeated mechanical plots would be beneficial.

The most effective treatments in reducing the cover of *R. caesius* were treatments that included the use of chemicals, as we expected. The chemical only treatment using Rodeo was effective in reducing the coverage and maintaining the reduction in coverage of *R. caesius* regardless of light environment. However, we did not have chemical only treatments at all the sites, so it would be beneficial to test this method further using additional sites. There was an extended period of reduced cover that continued after the second year of treatment application. Similar results were found by Delbart et al. (2012) who found that the combined mechanical and herbicide treatment of *R. japonica* was more effective in reducing the cover. They also found that chemical treatments can be more effective depending on the time of year *R. japonica* was treated due to the translocation of the herbicide to the rhizomes. Since *R. caesius* is also a rhizomatous riparian invader, it would be beneficial to try these treatments at different times of the year to determine if efficacy also depends on the timing of treatments.

Although the Rodeo herbicide was effective in reducing the cover of *R. caesius*, legal limits have been placed on the use of this herbicide due to environmental and health concerns. The combination mechanical and herbicide treatment using Garlon 4 Ultra was slightly more effective in the first round of treatments most likely due to its specificity to broadleaf plants and woody stems such as *Rubus*. However, this herbicide is not permitted for use near water. Instead of using these legally and environmentally restricted herbicides,
the treatment of mechanical removal twice is an option for the control of *R. caesius* as it was effective in reducing the coverage. This is important to note because land managers are often not permitted or the location if the invasion is not suitable for chemical use.

Interestingly, our data showed two outliers in two quadrats in the combined mechanical and herbicide-treated plots. These two quadrats had 78% higher coverage than the rest of the plots. The common trait of these two quadrats was that they were bordering a mechanical treatment plot. We can infer that during the mechanical treatments, a fragment was able to establish within the bordering combined treatment plot. The grass-cutting blade that was used left the fragments of *R. caesius* at less than an inch. Other *Rubus* species have been reported to be able to successfully grow from this size fragment (Barry 1988 and Best 1981), but our study (Chapter 1) investigated the growth from fragments of 10cm in length. Further research on these shorter fragments’ ability to grow would be needed. The fragmentation potential of *R. caesius* also raises concern for the locations mechanical treatments should be applied. For instance, mechanical treatments should not be applied to areas where the site is bordering or overhanging a riparian area to reduce the chance of spread down the watershed. Instead, hand-bagging or chemical treatments should be considered. Mechanical treatments would be better suited for locations not directly bordering a waterbody.

For future study, it would also be beneficial to investigate biological control mechanisms. Biological control is the reduction of pest populations by natural enemies or predators. An advantage to biological control mechanisms is that it saves the use of pesticides, which can be harmful to non-target species. However, there is risk concerning the introduction of exotic biological control agents with no predators to themselves (Babendreier
2007). *Rubus* species such as *R. alceifolius* have shown positive control responses to biological controls (Cybele et al. 2021). *Rubus alceifolius* is an invasive giant bramble present on Reunion Island. Cybele et al. (2021) found that *R. alceifolius* cover significantly decreased with the use of a defoliating sawfly, *Cibdela janthina*. However, they determined that the success of *C. Janthina* was influenced by altitudinal range, surface area of the patches, and the location of each patch (Cybele et al. 2021). Determining effective and safe biological controls for *R. caesius* would be beneficial, but extensive research is needed.
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mowing. Invasive Plant Science and Management 7(3): 140905130246003.


Tables:

Table 1. Description of sites and treatments: mechanical and herbicide using Rodeo (MH (G)), mechanical (M), repeated mechanical (Mx2), chemical using Rodeo (H(G)), and mechanical and herbicide using Garlon 4 Ultra (MH (T)) in low (L) and light (H) light conditions. Numbers indicate the number of each plot in each site.

<table>
<thead>
<tr>
<th>Site</th>
<th>Light</th>
<th>Control</th>
<th>MH (G)</th>
<th>M</th>
<th>Mx2</th>
<th>H (G)</th>
<th>MH (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>L</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>L</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>H</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>L</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>L</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>L</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>H</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
**Table 2.** Kruskal-Wallis test results for the treatments of mechanical, mechanical and herbicide using Rodeo, and control (no treatment) in low light conditions at sites A.B, C, D, and E.

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Kruskal-Wallis H</th>
<th>Df</th>
<th>Asymp. Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in Cover 2021</td>
<td>12.5</td>
<td>2</td>
<td>0.002</td>
</tr>
<tr>
<td>Change in Cover 2022</td>
<td>9.2</td>
<td>2</td>
<td>0.009</td>
</tr>
</tbody>
</table>
**Table 3.** Kruskal-Wallis test results for the treatments of mechanical, mechanical and herbicide using Rodeo, chemical only (Rodeo), repeated mechanical, and control (no treatment) at Site D in low light conditions.

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Kruskal-Wallis H</th>
<th>Df</th>
<th>Asymp. Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in Cover 2021</td>
<td>55.155</td>
<td>4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Change in Cover 2022</td>
<td>60.879</td>
<td>4</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Table 4. Kruskal-Wallis test results for the treatments of mechanical, mechanical and herbicide using Rodeo, chemical only (Rodeo), repeated mechanical, mechanical and herbicide using Garlon 4 Ultra, and control (no treatment) at Site E in high light conditions.

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Kruskal-Wallis H</th>
<th>Df</th>
<th>Asymp. Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in Cover 2021</td>
<td>47.264</td>
<td>5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Change in Cover 2022</td>
<td>.826</td>
<td>5</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Figure 1. Average change in cover of *Rubus caesius* from 2020 levels in the control (C), mechanical (M), and combination of mechanical and herbicide (Rodeo, MH) treatment plots in low light conditions. The year 2020 is pre-treatment. Replicates were five blocks. Errors bars indicate standard error. Different letters within a year indicate significant differences (p<0.05) among sites based on within year test pairs.
Figure 2. Average change in cover of *Rubus caesius* from 2020 levels in the control (C), mechanical (M), and combination of mechanical and herbicide (Rodeo, MH) treatment plots in high light conditions. The year 2020 is pre-treatment. Replicates were two blocks. Error bars indicate standard error.
Figure 3. Average change in cover of *Rubus caesius* from 2020 levels with site specific treatments of Herbicide only (Rodeo, H) and mechanical removal twice (M2) at Sites D in low light. N=10 sample quadrats in each plot. Error bars indicate standard error. Different letters within a year indicate significant differences (p<0.05) among sites based on within year test pairs.
Figure 4. Average change in cover of *Rubus caesius* from 2020 levels with site specific treatments of Herbicide only (Rodeo, H) and mechanical removal twice (M2), and the combination treatment using Garlon 4 Ultra (MHT) at Sites E in high light. N=10 sample quadrats in each plot. Error bars indicate standard error. The year 2020 is pre-treatment. Different letters within a year indicate significant differences (p<0.05) among sites based on within year test pairs.