ECOLOGICAL INVESTIGATION OF A POPULATION OF *TRILLIUM PUSILLUM* AT REDSTONE ARSENAL, ALABAMA

By

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A thesis submitted to the
Graduate faculty of Auburn University
in partial fulfillment of the requirements for
the Degree of Master of Science

Auburn, Alabama
May 4, 2019

Keywords: Redstone Arsenal, edge effects, life stage structure, seed production, deer herbivory, reproductive biology

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ABSTRACT

Trillium pusillum is a state-imperiled spring wildflower. Much information concerning its biology remains largely unknown. Four aspects of *T. pusillum* biology were investigated during this study. First, edge effects on life stage distribution were observed through comparative life stage counts among four belt transects. There were two edges onsite, one by a powerline and one by a road. Three transects were established at different distances parallel to the powerline edge and one transect was established parallel to the road edge. Counts of one-leaved, three-leaved, and reproductive plants were recorded three times a year for two years. Fruiting plants were counted as a subset of reproductive plants. T-tests were used to compare differences in counts between transects. Edge transects had higher percentages of reproductive plants than interior transects, which had higher percentages of one-leaved plants. Despite interior transects having smaller percentages of reproductive plants, plants in interior transects had the highest fruiting success. This study demonstrated edge effects on *T. pusillum* at this site. Second, deer herbivory was investigated via plant counts in ten pairs of *T. pusillum* clusters. Yearly plant count averages for both caged and uncaged clusters were compared via t-tests for 2016 through 2018. Plant counts did not differ significantly between caged and uncaged plots over the three years, showing no evidence of onsite deer herbivory. Third, seeds were buried in sleeves to test for a seed bank. Some seed sleeves were buried for two years while others were buried for three years. All buried seeds had germinated or rotted when they were unearthed in spring of 2018. Therefore, *T. pusillum* cannot retain a seed bank for more than two years. Lastly, some aspects of the pollination biology of *T. pusillum* were investigated, along with a survey of potential floral visitors. The pollination biology research consisted of a self-pollination study and a pollen-

supplementation study. In the self-pollination study, assisted selfing flowers produced a mean of 9.3 seeds per area and spontaneous selfing flowers produced a mean of 2.3 seeds per area. For the pollen-supplementation study, supplemented flowers produced a mean of 59 seeds per area while unsupplemented seeds produced a mean of 53 seeds per area. For both studies, t-tests showed there was no significant difference between mean seed counts of the treatments, showing that *T. pusillum* can produce seeds through selfing and that insect pollination of flowers produces as many seeds as pollen-supplemented flowers. One last t-test compared seed means between spontaneous selfing flowers and unsupplemented flowers. It revealed a significant difference, showing that self-pollinated flowers produce many fewer seeds than insect-pollinated flowers. Thus, insects pollinating *T. pusillum* are highly effective at pollination. The survey of potential floral visitors consisted of a study to document potential floral visitors (through pan and fermentation traps), along with a visual floral visitor survey. Nitidulid beetles visited flowers and were the most numerous specimens captured during the visual survey. These beetles also were captured in both trap types used during the potential floral visitor study. For these reasons, members of this genus might be pollinators of *T. pusillum* on this site. Future research might extend these studies to examine how edge effects may influence pollination success in *Trillium pusillum*.

ACKNOWLEDGEMENTS

I would like to acknowledge the help and support of the following individuals: Dr. Katherine Mincey, Jess Choi, Christine Easterwood, Brittany Harry, Bashira Chowdhury, Dr. Charles Ray, Gavin Shotts, Sunghun Jeong, Kaleigh Chalkowski, Dr. Todd Steury, Melanie Swick, Mark Swick, Alfred Schotz, and Marguerite McClintock.

Journal style used: Castanea

Software used: Microsoft Word version 15.35, Microsoft Excel version 15.35, R 3.3.3 GUI 1.69 Mavericks build (7328)

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I. INTRODUCTION

There have been five mass extinctions in the history of life (Raup and Sepkoski 1982). These include the Ordovician-Silurian (Sutcliffe et al. 2000), Devonian (Caplan and Bustin 1999), Permian (Twitchett et al. 2004), Triassic-Jurassic (Little and Benton 1995), and Cretaceous-Tertiary (Hallam 1987) extinction events. Mass extinctions are events in which over 75 percent of species are lost within a period of just over a million years (Barnosky et al. 2011). They can devastate both terrestrial and aquatic communities and change large tracts of landscape, including their biogeochemical components (Cao et al. 2009). Unfortunately, Barnosky et al. (2011) suggests that the planet may be in a sixth mass extinction. Smith et al. (1993) states that, over the past 400 years, approximately 486 animal and 600 plant species have gone extinct. A more recent report (Ceballos et al. 2015) examined the background extinction rate (natural extinction rate) of vertebrates versus the actual extinction rate. They estimated the actual vertebrate extinction rate is 100 times that of the background extinction rate. Many species of plants also have fallen victim to anthropogenic changes. Out of the estimated 450,000 species of angiosperms, two-thirds of these species, primarily tropical plants, are at high risk of extinction (Pimm and Joppa 2015). Pimm and Joppa (2015) state that the actual extinction rate for angiosperms may be anywhere from 1,000 to 10,000 times the background rate (which is one extinct species for every one million extant species every year).

Many factors could be influencing these declining population trends. Some plants are declining because of decreasing genetic variation (Loveless and Hamrick 1984). Decreasing effective population size, in some plant species, has caused a higher likelihood of inbreeding depression (Newman and Pilson 1997). Decreasing genetic diversity can then lead to local extinctions of plant populations across the globe (Matthies et al. 2004, Richards 1999). In

addition to genetic problems, habitat destruction accelerates plant extinction rates (Fortuna and Bascompte 2006). Dramatic changes in climate across the globe push more plant species closer to extinction (Fordham et al. 2012). Changing climate patterns impact entire regions and the habitats within them (Cowling and Lombard 2002).

Once a habitat is decimated, the effects of the event can last for centuries (Vellend et al. 2006). Food chains and food webs are disrupted, and trophic levels become less diverse as a result of habitat loss (Krauss et al. 2010). Interactions between species, such as pollination mutualisms between plants and insects, can be disrupted due to habitat disturbance. For example, Ashworth et al. (2004) showed that both generalist and specialist pollinators are highly vulnerable to extinction as a result of habitat disruption. Both habitat fragmentation and habitat degradation are leading causes of species decline and loss across the globe (Hoekstra et al. 2005, Kruess and Tscharntke 1994).

It is clear that human influence, over time, has caused higher rates of animal and plant extinction than would occur naturally. The single most important cause of these high rates of extinction is habitat destruction (Tilman et al. 1994), but habitat degradation also harms biodiversity hotspots and other sensitive habitat areas (Brooks et al. 2002).

One type of habitat degradation is habitat fragmentation: this occurs when a larger area of habitat is split into smaller patches (Haddad et al. 2015). A review by Wilcox and Murphy (1985) emphasized how fragmentation is linked to species loss. Fragmentation can either be viewed as spatial, when once continuous habitat is split into reduced fragments, or as constant degradation, when edge habitat constantly encroaches on a large tract of unfragmented interior habitat (Harrison and Bruna 1999). Smaller habitat patches that result from fragmentation often have higher extirpation rates than larger habitat patches (Loehle and Li 1996). Species with low

numbers and small geographic ranges, such as some mammals, are more susceptible to extinction through habitat fragmentation (Brooks et al. 2002). Oftentimes, genetic diversity can plummet due to fragmentation as a result of genetic drift within small, isolated, habitat islands (Young et al. 1996). Plant pollination relationships can be interrupted due to habitat fragmentation resulting in lower reproductive success in many plant species (Aguilar et al. 2006).

Fragmentation can negatively impact biodiversity in several major ways (Haddad et al. 2015). Three leading causes of biodiversity loss through fragmentation are decrease in habitat size, isolation of species, and adverse edge effects (Andren and Anglestam 1988). Not only can fragmentation and edge effects be detrimental to animals, they can also negatively impact plants.

Habitat fragmentation is a common cause of plant decline (Brooks et al. 2002). Disruption of important reproductive processes, such as pollination, is one way in which plant biodiversity loss is increased by fragmentation (Jules 1998). After fragmentation, plant-pollinator interactions can be severely disrupted due to a loss of plant species pollinated by specialist pollinators (Jules 1998). Even if fruits are produced, seed dispersal can be disturbed by habitat fragmentation (Bacles et al. 2006). Plants with seeds unsuitable for long distance dispersal may not be able to expand their populations if they become isolated by habitat fragmentation (Pearson and Dawson 2005). In addition, seed dispersal is an important mechanism of gene flow between habitat fragments (Bacles et al. 2006). Unfortunately, since seed dispersal patterns are highly dynamic, it may be difficult for biologists to quantify the effect of fragmentation on gene flow in plants (Cain et al. 2000).

Herbivory is another common challenge faced by many plants. High numbers of herbivores can decrease plant diversity (Olff and Ritchie 1998). For example, Olff and Ritchie

(1998) demonstrated that large concentrations of domesticated grazing mammals have caused loss of plant diversity in grassland habitat. Sometimes, herbivores may target plant parts on a species that may result in a decrease in that species' numbers. For example, white-tailed deer will consume the apical meristem or flowers of the *Lilium superbum* L. (Fletcher et al. 2001). Herbivory of this type can cause declines in both flowering and fruiting rates of some plant species (Augustine and Frelich 1998). It may take some plant communities over a decade to recover from the damage done by a year of intense deer browsing (Wilbur et al. 2017).

Another negative impact of habitat fragmentation is edge effects. Essentially, edge effects include negative biotic and environmental impacts that can damage interior species after fragmentation occurs (Kolb and Diekmann 2005). Interior species are species adapted to live in forest interior habitat rather than forest edge habitat (Villard 1998). Interior habitat is generally shadier due to the forest canopy, has lower temperatures, and is more uniform in terms of vegetative understory (Murcia 1995). In contrast, forest edge habitat has more sunlight, due to having reduced tree cover, and therefore has higher temperatures and higher amounts of low-growing plants (Murcia 1995).

Overall, edge effects can decimate interior species (Murcia 1995). Increased likelihood of disease, pest invasion, and changes in other biotic factors are only some of the impacts edge effects can have on plant communities (Murcia 1995). For example, edge effects can often be linked to herbivory. Deer herbivory tends to be greater near a forest edge (Cadenasso and Pickett 2000). Seed dispersal can be severely limited on forest edges due to increased seed predation (Lopez-Barrera et al. 2004). Lopez-Barrera et al. (2004) found that, in hard edges (edges where there is an abrupt, dramatic, change in vegetation), acorn consumption by rodents was much greater than on soft edges or in forest interiors. Johnson and Haddad (2011) examined how edge

effects can impact the transfer of plant fungal disease in corridors and fragmented forest patches. Plants that grew between 20 and 40 m from an edge were more likely to become infected, possibly due to air temperatures that may have been optimal for pathogen growth. Increased spillover of stinkbug pests in crop fields due to edge effects was observed in another study (Venugopal et al. 2015): populations of stinkbugs were significantly larger on field edges. Another study by Cadenasso and Pickett (2001) investigated the effect of forest edges on dispersal of seeds of invasive species into forest interiors. Their results indicated that forests with thicker edges of later successional stages were more likely to protect forest interiors against an influx of invasive seeds.

Edge effects can include various changes in the physical environment which can then impact plant populations. Microclimate factors that vary in edge habitat, such as canopy coverage and humidity, can affect forest communities (Gehlhausen et al. 2000). Gehlhausen et al. (2000) showed humidity was highly influential in determining differences in plant community composition between edge and interior habitats. Murcia (1995) showed that the number of new seedlings of several species was significantly less on edge than in interior locations, possibly due to higher air and soil temperatures on edges. Edge effects can even negatively impact plant population structure. Numbers of new *Trillium camschatcense* A. (Gray) seedlings were significantly fewer on the edge than in the interior, resulting in edge population structure consisting of older plants (Tomimatsu and Ohara 2003a).

Edge effects may impact life stage distribution of *Trillium,* a genus of understory plants found in East Asia and North America (Tomimatsu and Ohara 2003a). These edge effects include changes in seedling recruitment, seed predation, and microclimatic changes. For example, seed recruitment was negatively impacted by low seed production for *Trillium ovatum*

plants found near forest edges, and seedling recruitment decreased dramatically (Honnay et al. 2005). *Trillium camschatcense* seedlings, observed in another study, were not as common on the edges as they were in the interior (Tomimatsu and Ohara 2003a). Seed predation by animals, such as mice, was greater for *Trillium camschatcense* plants growing on forest edge habitats (Tomimatsu and Ohara 2003a). Seed predation by deer mice also decreased seedling recruitment in *Trillium ovatum* (Tallmon et al. 2003).

Microclimatic differences, such as air temperature, between edge and interior habitat have been suggested as additional explanations for decreased seedling recruitment on edges (Gehlhausen et al. 2000). Tomimatsu and Ohara (2003a) investigated how edge effects impacted seedling recruitment in *Trillium camschatcense* populations, suggesting that seedling establishment was reduced by higher temperatures on the forest edge. They also suggested that low seed production was due to lower numbers of forest interior pollinators (such as beetles) on edges (due to higher temperatures on edges). As a result, recruitment of young plants was significantly lower on the forest edge. Other species of *Trillium*, such as *Trillium ovatum*, have experienced decreased seed production due to decreased pollination success under edge conditions (Jules and Rathcke 1999).

The genus *Trillium* is a member of the family Melanthiaceae found in North America and East Asia (Case and Case 1997, Chauhan et al. 2018). There are two groups of *Trillium*: 22 sessile-flowered species and 29 pedicellate-flowered species (Ohara and Utech 1986). Members of this genus have both leaves and petals in whorls of three (Gates 1917). Flowers are typically white, pink, or red, but can come in shades of green or yellow (Kawano and Kato 1995). Leaves are typically simple and ovate and may either be solid-colored or blotched (Gates 1917). The stem of *Trillium* plants is a segmented, starch-filled rhizome that can produce multiple shoots

(Vellend 2005), often leading to clonal clusters of *Trillium* plants (Gonzales et al. 2008, van Kleunen et al. 2001). Plants in this genus can reproduce clonally or sexually, depending on habitat conditions (Gonzales et al. 2008). Clonal reproduction is thought to be more common in habitats where there is more competition for resources such as space and moisture (Gonzales et al. 2008). Clonal reproduction occurs by lateral budding from the rhizomes (Nesom and La Duke 1985). Habitat varies from hummocks in swampy, lowland forests to mountainsides and granite outcrops (Case and Case 1997, Farmer 2006, MacRoberts and MacRoberts 2005). In the Eastern United States, *Trillium* typically grows in forest interiors populated primarily by oak, poplar, and beech (Ohara and Utech 1986).

Trillium are long-lived and can take over a decade to mature (Case and Case 1997). They undergo four stages during their lifetimes (Yamagishi et al. 2007). The first (seedling) stage begins with germination and continues for several years (Kawano et al. 1992). This is followed by a one-leaved immature stage in which a single leaf at the tip of the stem has fully unfurled. A three-leaved immature plant is the next stage in which the whorl of three leaves is fully formed. Finally, a plant at the mature stage produces a flower (Kawano et al. 1992).

Trillium flowers generally are pollinated by insects (Case and Case 1997). Coleopterans are an insect group reported to pollinate some *Trillium*, such as Nitidulidae pollinating *T. camschatcense* (Tomimatsu and Ohara 2003b). Other insects such as moths and dipterans have been shown to visit flowers of some *Trillium* species, however, it is unknown if these insects act as pollinators (Davis 1981, Knight 2004). *Trillium catesbaei* is mainly pollinated by queen bumblebees (Motten 1986). Some *Trillium* species, such as *T. erectum*, are capable of self-pollination (Davis 1981). Others, such as *Trillium grandiflorum*, are self-incompatible (Steven et al. 2003, Heckel and Leege 2007).

Trillium seeds are encased in small yellow or green fruits and are attached to a lipid-filled food body called the elaiosome (Pavlovic et al. 2014). Seeds are often dispersed by ants that use the elaiosomes as a food source due to their high oil content (Pavlovic et al. 2014). Once seeds are dropped by ants, they may remain underground in ant burrows for the duration of their double dormancy, and clusters of new seedlings may emerge (Gunther and Lanza 1989).

Once new plants are established, deer herbivory can be a detriment to *Trillium*. Herbivory of *Trillium* meristems by white-tailed deer may cause life stage regression. For example, reproductive plants can regress back to non-reproductive stages as a result of being partially consumed by deer (Barfield et al. 2011). White-tailed deer tend to prefer flowering *Trillium* plants and older immature non-flowering plants rather than seedlings (Knight et al. 2008). Webster et al. (2015) showed that deer herbivory adversely affected reproductive success of *Trillium catesbaei*, as populations exposed to high levels of deer herbivory were far less successful due to the consumption of reproductive plants than those that were not subject to deer herbivory. Herbivory by white-tailed deer can drastically reduce seed production of mature *Trillium* plants in edge habitats (Opperman and Merenlender 2000). Typically, reproductive plants consumed in the early days of the growth season will grow much smaller and regress to a non-reproductive life stage (Knight 2003).

Study Species

Trillium pusillum Michx. is a small, understory perennial native to North America (Case and Case 1997). Its range extends throughout the southeastern United States where it is subdivided into several taxa forming the *Trillium pusillum* Michaux Complex, recognizing French naturalist Andre Michaux who first described the species in the Carolinas (Roe 1978).

Taxa include *Trillium pusillum* Michx. var. *pusillum* in the Carolinas, *T. p.* Michx. var. *virginianum* for the Virginias, *T. p.* Michx. var. *ozarkanum* in Arkansas and Missouri, *T. p.* Michx. var. *monticulum* in Virginia and West Virginia, and *T. p.* Michx. var. *texanum* in Texas (Timmerman-Erskine et al. 2002, Weakley 2015). A number of other *T. pusillum* taxa exist, including varieties *T. p.* Michx. var. *carolinianum, T. p.* Michx. var. *georgianum, T. p*. Michx. var. *palustris*, and *T. p.* Michx. var. *telmacola* (Weakley 2015). Another taxon is *T. p.* Michx. var. *alabamicum* (Weakley 2015). The name *alabamicum* was given because this variety occurs mainly in Alabama and occasionally in Kentucky and Tennessee (Case and Case 1997, Timmerman-Erskine et al. 2002). This taxon is currently classified by Weakley (2015) within *T. p.* var. *pusillum*. NatureServe Explorer (2017) states that all taxa of *T. pusillum* are considered to range from vulnerable to critically imperiled. *Trillium p.* var. *pusillum* occurs in Alabama within Franklin, Lawrence, Morgan, and Pickens Counties (Nature Serve Explorer 2017). According to NatureServe Explorer (2017), *T. pusillum* has a state conservation rank of S2 in Alabama, meaning that it is imperiled with only six to twenty populations statewide.

Flowers of *T. pusillum* possess petals that are either white or a light pink (Kawano and Kato 1995). It is unknown what animal species may pollinate *T. pusillum*, if any. Fruits of *T. pusillum* are fleshy and drab-colored (Cabe and Werth 1995). Fruits are capsules with a globose ovary (Kawano and Kato 1995). As in other *Trillium* species (Pavlovic et al. 2014), the seeds bear an oily elaiosome. Leaves of *T. pusillum* are lanceolate and green with a purplish underside (Kawano and Kato 1995). *Trillium pusillum* is typically found in the interiors of lowland, woodland swamps and riparian areas (Bodkin and Reveal 1982).

Goal of Thesis:

The goal of this thesis is to investigate ecological aspects of *Trillium pusillum*. The study is divided into four components: edge effects, deer herbivory, seed bank, and pollination biology. The first component attempts to document any impact of edge effects on *T. pusillum* life stage distribution or fruiting success. Edge effects data include the relative numbers of the various plant life stages at different distances from the forest edges. Evidence that fragmentation may pose a threat to life stage distribution and reproduction of *T. pusillum* could be uncovered. The second component will determine if deer herbivory occurs on *T. pusillum*. Deer exclosure data can alert habitat managers and biologists if there is any potential danger of white-tailed deer herbivory to the *T. pusillum* population at Redstone. Third, seed burial will test if *T. pusillum* is able to retain a seed bank. Lastly, pollination biology is split into two main components. First, one component will test seed production in insect pollinated and pollen-supplemented flowers, as well as in self-pollinated flowers. The second component includes trapping potential insect floral visitors and a floral visitor visual survey. Data collected from each component of the four study components can potentially aid in protecting and conserving the population of *Trillium pusillum* at Redstone Arsenal and other populations. Managers can potentially utilize these results to better understand how reproduction of this species is carried out for possible, future population studies.

II. MATERIALS AND METHODS

Site Description

This study took place in a swampy lowland forest within the boundaries of Redstone Arsenal in Madison County, Alabama. Coordinates of the study site are 34° 38' 48.88" N and 86° 37' 13.97" W. It was approximately 0.8 hectares in total area and was bordered on the north side by a four-lane road and on the east side by a powerline right-of-way that was approximately 30 m wide (Figure 1). Elevation of the study site was around 180 m above sea level. Soil was gray in color and covered by standing water, primarily in March and early April. Dominant tree species included hardwoods such as *Quercus alba* L. and *Carya ovata* K. Koch. while midstory trees included *Aesculus pavia* L. and *Carpinus caroliniana* Walter. Other understory forbs, aside from *Trillium pusillum*, were sparse in the interior but were dense near the road edge and powerline right-of-way. The road and the powerline were the only human-made structures within 0.5 km of the study site.

There were two types of edge on the study site (Figure 1). The Powerline Edge was approximately four years old in 2018. The powerline has been in existence for much longer, but the edge was moved farther into the forest in 2014 when trees were cut down to widen the powerline right-of-way. The Road Edge was more than 15 years old at the time of the study. The Road Edge was treated with herbicide annually to prevent plant growth on the rip-rap (limestone boulders) that borders the pavement next to the site. To date, the Powerline Edge has not been treated with herbicide.

The vegetation varied between both edges. Young hardwoods such as *Liriodendron tulipifera* L. and *Salix nigra* Marshall, and evergreens such as *Juniperus virginiana* L.,

surrounded by *Vitis rotundifolia* Michx. and *Rubus* sp., grew within the Road Edge. Plants such as *Smilax* sp., *Andropogon virginicus* L., *Aralia spinosa* L., *Aesculus pavia* L., and *Acer rubrum* L. populated the Powerline Edge. Patches of *Toxicodendron radicans* (L.) Kuntze were found in both edge habitats.

The forest interior was dominated by tree species such as *Acer rubrum, Quercus alba,* and *Fagus grandifolia* Ehrh. Undergrowth was sparser than on the edges and dominant species included *Vitis rotundifolia*, *Smilax* sp., and *Aesculus pavia*. The areas of forest interior, closer to the road, held standing water from March to early April.

Study I Methods: Edge Effects and Life Stage Distribution of *Trillium pusillum*

Belt transects were established to allow sampling of the *T. pusillum* population in both edge areas, as well as at two interior sites (Figure 1). Four belt transects were included in this study. One was parallel to the powerline, two were interior and parallel to the powerline, and one was arranged parallel to the road edge. The three belt transects parallel to the powerline edge were each 5 m wide. The Powerline Edge transect was placed inside the forest edge immediately bordering the powerline right-of-way. A second transect (Interior 1) was located 20 m into the woods from the edge, and a third (Interior 2) was placed an additional 20 m into the woods (40 m total from the edge). Each of these three belt transects measured approximately 200 m long by 5 m wide. A fourth transect, the Road Edge transect, was established parallel to the road immediately inside the edge of the forest. In 2017, it measured 75 m by 5 m and was widened to 75 m long and 15 m wide in 2018 to include a larger sample size of *T. pusillum*. It was wider than the other transects because its length (which encompassed the edge containing *T. pusillum* plants) was shorter. The four transects served to create a manageable data collection area (about

1,000 m^2 in each) within each area of the study site. They were marked with flagging tape, nylon flags, and plastic tent stakes.

Numbers of *T. pusillum* plants in each life stage (one-leaved immature, three-leaved immature, and reproductive) were counted in each transect during the springs of 2017 and 2018. Data were collected on April 1, 8, and 21 in 2017, and on March 15 and 30, and May 5 in 2018. Seedlings were excluded from the list of life stage categories because they could not be reliably identified due to their resemblance to other small understory plants. On May 5 of both years, numbers of fruiting plants were counted in order to determine fruiting success of all reproductive plants in each transect.

Comparisons were made of relative numbers of each of the three life stages in the belt transects for each of the two years using contingency table analysis. A Chi-square test in R, version 3.3.3 GUI 1.69 Maverick's build (R Foundation for Statistical Computing 2016), with a four-by-three table (four transects, three life stages), was used to analyze the entire dataset from each year to determine if there was significant variation in numbers of plants in each of the three life stages among all transects. Pairwise Chi-square tests were then used to compare all pairs of transects against each other. For example, 2017 life stage data were compared between the Road Edge transect and the Interior 1 transect, the Road Edge transect and the Powerline Edge transect, and so on.

Fruiting success was determined on May 5 each year when numbers of flowering plants that produced fruits were counted for each transect. Contingency table analysis in R, version 3.3.3 GUI 1.69 Maverick's build (R Foundation for Statistical Computing 2016), was then used to compare fruiting success of reproductive plants in the transects. For the data in each year, contingency table analysis, using a four-by-two table (four transects, and the two conditions of

fruiting and non-fruiting), was used to compare fruiting success for all four transects. If a significant difference was found in fruiting success for the four transects in a given year, pairwise contingency table analyses of fruiting success between each possible combination of transects were executed to find where the significant differences lay.

Study II Methods: Deer Herbivory of *Trillium pusillum*

In the deer herbivory study, wire cages were used to protect selected *Trillium* clusters from foraging white-tailed deer. Cages were made from green plastic-coated metal wire mesh with rectangular holes that measured two by seven centimeters. The cylindrical cages were 0.8 m tall by 0.4 m wide and closed at the top. Ten cages were set up on the study site on March 27, 2016. Each cluster of caged plants was paired with a nearby uncaged cluster. Pairs of clusters were selected by locating two dense clusters of *T. pusillum*, each containing at least one flowering plant, that were relatively close to each other. A coin was flipped to randomly determine which cluster would be caged and which would be uncaged. Numbers of plants at each life stage were counted for both the caged and uncaged treatments three times per season. From those three data sets, the highest count of each life stage (per cluster) was compiled into a summary data set. Numbers of each life stage (one-leaved immature, three-leaved immature, reproductive) per cluster in the summary data set were then added to calculate a total yearly plant count per cluster.

A generalized linear mixed model, glmerMod in the lme4 package, was executed in R version 3.3.3 GUI 1.69 Maverick's build (R Foundation for Statistical Computing 2016) to compare yearly plant totals of all three years (2016, 2017, and 2018). The model contained a Poisson error structure due to total plant counts being recorded as integers. Block, or plant

cluster pair, was used as a random effect while cage presence was used as a fixed effect. The cage presence factor was included to detect if total plant counts varied significantly between caged and uncaged clusters. Significance of the cage presence factor would be evidence that herbivory may have occurred during the study.

Study III Methods: Evidence of a *Trillium pusillum* Seed Bank

For a seed bank to be formed, *Trillium pusillum* seeds must remain dormant beyond at least one spring when buried in the soil. Harvest of seeds for this study took place in both 2015 and 2016. Seeds were placed in seed sleeves, which were envelopes that protected seeds from predators during the burial period. Sleeves were constructed with two layers. The first (outer) layer was made from plastic, 4-hole-per-centimeter window screen, which protected seeds from predators, and a second (inner) layer was made from 12-hole-per-centimeter no-see-um netting, which held seeds in place. Sleeves were closed with staples after seeds were deposited.

Seeds were collected and, on the day of collection, placed in seed sleeves. On June 19, 2015, three seed sleeves containing five seeds each were buried, and on June 25, 2016, four sleeves containing ten seeds each were buried. Seed sleeves were buried 1 cm deep in four adjacent spots along a transect marked with PVC piping and pin flags. Burial locations were spaced about 1 m apart from each other and were located in the forest approximately 10 m from the powerline right-of-way. Seeds from both 2015 and 2016 were uncovered in June 2018, after three years and two years of burial, respectively, to count any remaining seeds.

Study IV Methods: *Trillium pusillum* Pollination Biology

This section of the study is split into three subsections. Each provides information useful for understanding the pollination biology of *Trillium pusillum*. They include a selfing study, a pollen supplementation study, and a study to document potential floral visitors on this site.

The selfing study took place in locations outside of the belt transects beginning on March 30, 2018. Thirty pairs of plants with flower buds were selected. These were located in three plots of ten pairs adjacent to each of the belt transects used in the Edge Effects and Life Stage Distribution Study. White polyester organza bags, of mesh size 0.2 mm by 0.2 mm, were placed over each plant and supported by bamboo stakes so that plants would not be weighed down (especially during rain events). Although entire plants were bagged, sunlight could still enter the bags due to the transparency of the fabric. A coin was flipped to determine which pair member received the assisted selfing treatment. Assisted selfing was accomplished by removing the bag, dusting the flower's own pollen onto its stigma with a fine-tipped paintbrush, and then re-bagging the plant. The second plant in each pair was not self-pollinated and served as a control to determine whether self-pollination could occur spontaneously. Bags remained on all plants throughout the experiment. Pollination treatments were done on March 30, and again on April 2, 2018.

Seed production was compared between assisted selfing and spontaneous selfing treatments by analyzing mean seed counts for each treatment in each area. Seeds were collected on April 21, 2018. The total number of seeds produced was counted for each treatment from each of the three plots. These numbers were then analyzed using a t-test in R version 3.3.3 GUI 1.69 Maverick's build (R Foundation for Statistical Computing 2016) to determine if assisted selfing increased seed production compared to spontaneous self-pollination.

For the pollen supplementation study, thirty pairs of flowering plants inside the transects were chosen on March 30, 2018. Ten pairs of plants were chosen within each transect and marked with flags. One plant pair member was supplemented with a pollen donation from plants outside of the belt transects, while the other pair member was unsupplemented by donor pollen. A coin was flipped to determine which plant received each treatment. Pollen donor plants were more than 5 m away from the plant pairs, and their anthers were clipped off and placed in a petri dish. Pollen was deposited on the selected pollen-supplemented flower with a piece of fishing line on March 30 and April 2, 2018.

As with the selfing experiment, mean seed counts per treatment per area were calculated for the pollen supplementation experiment. Total numbers of seeds produced were counted on April 21, 2018, for each treatment from each transect of the pollen supplementation study. T-tests using R, version 3.3.3 GUI 1.69 Maverick's build (R Foundation for Statistical Computing 2016), tested treatment effects on mean seed count.

In addition, a t-test was performed to compare seed counts per area of bagged unmanipulated flowers versus open unmanipulated flowers. This test compared seed production between pollinator exclusion and naturally pollinated flowers to determine whether spontaneous self-pollination or insect pollination was more effective at producing seeds.

The final study was separated into two components. First, a field survey was conducted to observe floral visitors to *T. pusillum* at peak flowering time. Visual surveys of flowering plants were completed during peak flowering on the site, on March 14 and 16, 2018, to capture insects visiting *T. pusillum* flowers. Insects observed on flowers were captured, counted, and separated according to order and family. Second, both pan and fermentation traps were used to capture potential floral visitors on site. The pan trap study was conducted on March 17-18, March 24-25,

and April 2-3, 2018. Fermentation trapping was done on March 17-18 and again on March 24-25, 2018. Pan traps were constructed from blue or yellow plastic bowls because many insect pollinators are attracted to these colors (Campbell and Hanula 2007). Six pan traps, three of each color, were set 5 m apart along each of the Powerline Edge, Interior 1, and Interior 2 transects. Water and dish soap were used to fill the pans in order to capture insects. After 24 hours, trap contents were collected into plastic jars, separating the specimens according to pan color and belt transect. Insects in each sample were then identified to order and family, and the numbers of individuals counted. Six fermentation traps were constructed from 500 ml plastic containers and apple cider vinegar was poured inside to attract insects. Traps were placed above clusters of blooming *T. pusillum* by hanging them from tree branches or snags in order to capture insects in the vicinity of *T. pusillum* flowers. Two fermentation traps were placed on the Powerline Edge, Interior 1, and Interior 2 transects. As with the pan traps, fermentation traps were left for 24 hours before their contents were collected for insect sorting, identification, and counting.

III. RESULTS

Study I Results: Edge Effects and Life Stage Distribution of *Trillium pusillum*

Transects varied in plant counts. In both years, the greatest total number of plants was found in the Interior 1 transect (908 in 2017 and 1,206 in 2018) (Figure 2). The Road Edge had the smallest counts of plants (77 in 2017 and 413 in 2018) (Figure 2). In 2017 the Road Edge transect's area was 375 m^2, as opposed to the other three transects (which were 1,000 m^2), and this small area explains the lower plant counts. However, the Road Edge transect was expanded in 2018 to an area of 1,125 m^2 to include a larger sample size.

Life stage composition differed significantly between transects. The four-by-three Chi-square test for 2017 showed a significant difference between the life stage counts of all four transects (Pearson's Chi-square test, Chi-square = 80.6, df = 6, $p < 0.05$). In 2018, the four-by-three Chi-square test also revealed significant differences in life stage composition (Chi-square = 73, df = 6, $p < 0.05$). Most pairwise comparisons among transects within a year were also statistically significant (Table 1): the exceptions being Interior 1 vs. Interior 2 (in 2017 and 2018) and Powerline Edge vs. Road Edge (in 2017). Over all of the transects, for both years, there were higher percentages of one-leaved plants in the interior transects (Figure 3). Furthermore, in both years, there were higher percentages of reproductive plants in edge transects (Figure 3). These differences indicate potential edge effects on this site.

Fruiting success varied significantly among the four transects in both 2017 (Chi-square = 33.5, df = 3, $p < 0.05$) and 2018 (Chi-square = 55.5, df = 3, $p < 0.05$). Most pairwise comparisons revealed significant fruiting success differences between transects (Table 2). Exceptions for 2017 included Interior 1 vs. Interior 2, Interior 1 vs. Road Edge, and Interior 2 vs. Road Edge (Table

2). In 2018, the only non-significant fruiting success comparison was Powerline Edge vs. Road Edge (Table 2). There were distinct differences in fruiting success in both years between edge and interior habitats (Figure 4). Overall, interior transects had significantly higher fruiting success than edge transects.

Study II Results: Deer Herbivory of *Trillium pusillum*

Most plants (including caged plants) did not appear to have been damaged in any year. Only one uncaged plot, close to the forest edge in mid-April of 2018, contained plants that may have been consumed by deer. Flowers were gone, and the tops of the stems appeared to have been eaten. However, it was not determined whether they had been consumed by deer or insects. The mixed effects model in R showed that the cage presence factor did not significantly affect total plant counts per cluster ($F = 2.5$, $df = 1$, $p = 0.11$). Yearly mean plant counts, for both caged and uncaged clusters, were similar across all three years (Figure 5). These results indicate no immediate danger of deer herbivory on site.

Study III Results: Evidence of a *Trillium pusillum* Seed Bank

No dormant seeds were left after the two or three-year burial periods. Seed sleeves did not contain any seeds when uncovered in June 2018. Seeds most likely either germinated or rotted while in the sleeves as opposed to falling victim to underground seed predators, as seed sleeves did not appear to be damaged when they were uncovered. Therefore, *T. pusillum* is not able to retain a seed bank for over two years.

Study IV Results: *Trillium pusillum* Pollination Biology

Flowers in both treatments of the selfing study produced fruits. Several plants senesced before they could produce a fruit because they were weighed down by the bag and smothered in mud or detritus. There was no significant difference between mean seed counts of assisted selfing and spontaneous selfing flowers ($t = -1.1$, $df = 1$ $p = 0.33$). Assisted selfing flowers produced an average of 9.3 ± 5.8 (mean ± SE) seeds per treatment per area while spontaneous selfing flowers produced an average of 2.3 ± 2.3 seeds per treatment per area.

Pollen-supplemented plants showed a similar lack of difference between treatments. These flowers produced a mean (± SE) of 59 ± 11 seeds per treatment per area and unsupplemented plants produced 53 ± 9.5 seeds per treatment per area. These means were not significantly different ($t = -0.43$, $df = 1$ $p = 0.69$).

Pollen supplementation study flowers produced more seeds than flowers in the selfing study. A final statistical comparison, between unmanipulated plants in both studies, revealed a significant difference between seed counts due to pollinator exclusion ($t = -5.2$, $df = 1$ $p = 0.0066$). Mean seed count per treatment per area (± SE) for pollen unsupplemented (insect pollinated) flowers was 53 ± 9.5 and only 2.3 ± 2.3 for spontaneous selfing flowers.

Overall, the visual floral visitor survey revealed two orders of insects visiting *T. pusillum* flowers. A total of 20 specimens in the orders Coleoptera and Diptera were captured. Within Coleoptera, Nitidulidae (sap beetles) were the most common floral visitors to *Trillium pusillum*. Ten specimens of Nitidulids in the genus *Meligethes* sp. were collected. Individuals of *Meligethes* sp. were captured across the site during the second survey, on March 16, 2018. One Nitidulid not in the genus *Meligethes* was captured, along with one specimen each of Curculionidae and Staphylinidae. A total of seven Dipterans were captured. Chloropidae was the

most common family within that order, with a total of three specimens. One specimen was captured of each of the following families: Emphididae, Phoridae, and Scatopsidae.

Surveys of potential insect floral visitors revealed several major insect orders on the site. Overall, most insects were captured in pan traps and the highest insect counts were found on the Interior 1 transect for pan traps (Figure 6a) and the Interior 2 transect for fermentation traps (Figure 6b). Pan traps collected a greater number of insect orders than fermentation traps, which collected two main orders, Diptera and Coleoptera (Figure 6). Within the fermentation traps, Drosophilidae (pomace flies) was the most common family in Diptera and Nitidulidae (sap beetles) was the most common family within Coleoptera (Table 3). Within the most common orders found in the pan traps (Coleoptera, Diptera, Hemiptera, and Hymenoptera), there were several common reoccurring families (Table 3). Buprestidae (jewel beetles), Carabidae (ground beetles), and Nitidulidae were the most common families within Coleoptera. Chironomidae (non-biting midges) and Dolichopodidae (long-legged flies) were the two Dipteran families with the highest counts. Aphidae (aphids) and Cicadellidae (leafhoppers) were the most common families found in Hemiptera. Lastly, Ichneumonidae (ichneumon wasps), and Microhymenoptera (a general term for small Hymenopterans), were the two most common taxa under Hymenoptera (Table 3). Megachilid and Halictid bees, which were the only bee families captured on the Redstone study site, were found mainly on the edge transects. One last order, Collembola (springtails), was obtained from the pan traps but individuals were too numerous to count and so are not included in Figure 6.

IV. DISCUSSION

In each of the four components of this study, new information was uncovered about the biology of *Trillium pusillum*. In Study I (Edge Effects and Life Stage Distribution of *Trillium pusillum*), life stage distribution differed among transects. Overall, the edge transects had greater percentages of reproductive plants than the interior transects. *Trillium* plants typically show greater percentages of one-leaved plants in forest interior habitat rather than in edge areas (Marchand and Houle 2005). Studies of other plants, such as *Peraxilla tetrapetala* (L.f.) Tiegh in New Zealand (e.g., Burgess et al. 2006), have shown that both higher flowering and fruit set can occur on the edges of fragmented habitats. One possible explanation for higher percentages of flowering on the edge may be increased light levels. For example, *Trillium erectum* may need more direct sunlight in the early spring in order to produce more flowers (Routhier and Lapointe 2002). On my study site, *Trillium pusillum* on the forest edge might have been responding to greater light levels.

Despite greater percentages of reproductive plants, plants in edge transects had lower fruiting success than those in interior transects. This can be due to drastic differences in pollinator species composition between different habitat types (Kearns and Inouye 1997). Some pollinators tend to pollinate either on the edge or in the interior. For example, meliponine bees are not typically found pollinating flowers in forest interiors (Brosi et al. 2008). Similarly, Bailey et al. (2014) showed that bee species density and richness decreased at increasing distance from a forest edge. Another study showed that pollinators in the genus *Apis* tended to decrease in numbers at approximately 500 meters inward from a forest edge (Chacoff and Aizen 2006).

Perhaps the pollinators of *T. pusillum* were not as abundant on edge transects as they were on interior transects.

Non-reproductive plants showed distinct distribution patterns as well. For both years, one-leaved plants were more numerous in the interior (Figure 3). These one-leaved plants can include young *T. pusillum* as well as any regressed individuals. There are three possible explanations for the higher numbers of one-leaved plants on the interior. These are clonal growth, regression due to herbivory, and higher seedling recruitment.

Trillium pusillum is clonal, so even if seedlings are scarce new individuals can arise from rhizomes (Cabe and Werth 1995). In this case, clonal reproduction in the interior can possibly account for the high numbers of one-leaved plants. A study on *Trillium* ramets sought to explore clonal growth in non-reproductive ramets of *Trillium recurvatum* (Moore et al. 2012). Their stem census data revealed that non-reproductive ramets had higher growth rates than reproductive ramets. However, they did not mention if these non-reproductive ramets gave rise to more one-leaved or three-leaved plants. It is unclear whether clonally-produced *Trillium* plants begin at the one-leaved or three-leaved life stage. This would make it difficult to tell if the one-leaved plants on the interior transects at the Redstone site arose from clonal growth.

Clonal growth might also have been influenced by abiotic habitat factors that varied between edge and interior areas. Moore et al. (2012) observed fluctuations (possibly due to microclimatic changes such as temperature and moisture) in clonal ramet growth and non-reproductive plant recruitment over a seventeen-year census period. This could possibly contribute to higher numbers of one-leaf plants in the interior, as perhaps the microclimate in the interior was more optimal for clonal growth and led to greater numbers of recently-produced one-leaved plants.

Deer herbivory, which could cause *Trillium* plants to regress to non-reproductive stages, was not present on this site, so regression is not a likely explanation (Barfield et al. 2011). Therefore, the one-leaved plants on the interior transects probably are younger plants, or slow-growing plants, rather than regressed individuals. On other sites, that have higher levels of deer browse, *T. pusillum* regression due to herbivory may be a factor.

However, another possible explanation could be higher seedling recruitment on the forest interior versus the edge, if the majority of one-leaved plants in the interior are young plants produced from seeds. *Trillium ovatum* was shown to have reduced seedling recruitment, and therefore fewer one-leaved plants, when experiencing edge effects (Jules and Rathcke 1999). Both decreased resources and increased seed predation in edge habitat were given as reasons that seedling recruitment would be lower (Jules and Rathcke 1999). Future studies of edge effects on *Trillium pusillum* could measure microclimatic factors and make comparative seedling counts to further explore reasons behind the differing life stage makeup of edge and interior habitats.

No current threat of deer browsing was found for *Trillium pusillum* at this Redstone Arsenal site. However, deer browsing might negatively impact *T. pusillum* in other areas of its range. Deer browse was shown to cause declining growth in a population of *T. grandifolium* (Rooney and Gross 2003). Both reproductive and non-reproductive plants in three species (*T. reliquum, T. cuneatum,* and *T. maculatum*) were consumed by white-tailed deer (Leege et al. 2010). Anderson (1994) suggested that *Trillium* is an effective deer browse indicator, as height of *Trillium grandiflorum* was negatively correlated with the amount of deer browse. This relationship can assist land managers in assessing deer browse damage in habitats that may appear undisturbed by high deer density (Anderson 1994). Future studies at other *T. pusillum*

habitat sites could potentially use yearly plant height measurements as an indicator of deer herbivory damage.

No evidence was found that *Trillium pusillum* formed a long-lived seed bank. No seeds were left when all of the seed sleeves were uncovered, showing that *Trillium pusillum* seeds are most likely not able to form a seed bank older than two years. Double dormancy requires two years to complete (Kondo et al. 2011). Another study (Brown 1992) involving an unnamed *Trillium* species showed that it did not create a seed bank. When soil samples were taken from *Trillium* habitats with known seed banks of multiple species (including *Verbascum thapsus* L.), none of the sprouted seedlings belonged to the genus *Trillium* (Brown 1992).

Trillium pusillum seed production depended on pollination. Pollen-supplemented, and insect pollinated, flowers had greater seed counts than selfed flowers, showing that on-site pollinators were effective in maximizing *T. pusillum* seed production. However, *T. pusillum* was able to produce a few seeds through selfing. *Trillium camschatcense* also has been shown to produce seeds via self-pollination (Tomimatsu and Ohara 2006). For *Trillium grandiflorum*, self-pollinated flowers also produced fewer seeds than pollen-supplemented flowers (Steven et al. 2003).

During the floral visitor study, several findings were pertinent to the question of which insects pollinate *Trillium pusillum*. However, there are still no definite answers as to the species that may pollinate *T. pusillum*. *Trillium grandiflorum* is pollinated by *Bombus* sp. (Knight 2004) and Ohara and Kawano (2005) report that *T. camschatcense* is pollinated by both Hymenopterans (*Andrena* sp. and *Bombus* sp.) and Coleopterans (*Aphthonaltica* sp. and *Carpophilus* sp.). Both of these aforementioned insect orders were captured in pan traps on site but were not observed pollinating *Trillium pusillum*.

More seems to be known about insects that simply visit flowers of the genus *Trillium* as opposed to insects that pollinate these plants. Coleopterans, Dipterans, and Hymenopterans have been reported to visit both *T. cuneatum* and *T. grandiflorum* (Shipman 2011). Dipterans were the most common flower visitors, especially the families Sciaridae and Mycetophilidae (Shipman 2011). *Trillium grandiflorum* was mainly visited by Hymenopterans (primarily *Apis* and *Bombus*), and *T. erectum* was as well (Irwin 2000). Insect visitors were documented carrying *Trillium* pollen, but it was not known if this pollen was deposited on the stigmas of plants (Irwin 2000). However, members of the family Nitidulidae, within the order Coleoptera, are reported as floral visitors to *Trillium* (Tomimatsu and Ohara 2003b). Members of the Nitidulidae, species *Meligethes*, were captured in both the pan and fermentation traps at the Redstone site. Members of this family were the most numerous insects captured during the visual floral visitor survey of *T. pusillum*.

Future studies could utilize field observations to catalogue floral visitors. Before field observation takes place, lists could be compiled of known *Trillium* pollinators in the study area, so that a set of expectations may be made as to which insects could be observed during the study (Tomlinson et al. 1979). A study on the biology of *Apios americana* Medik. used a "single visit" technique to compare the pollination efficiency of floral visitors (Paris and Boyd 2018). Future studies could incorporate this method for *T. pusillum*.

Overall, this thesis presents several findings for *Trillium pusillum* biology. First, edge effects impact this plant's life stage distribution and fruiting success. Second, while no deer herbivory was present at the Redstone site, it may be present in other *T. pusillum* habitat sites. Third, no seed bank is present beyond the two-growing-season period of double dormancy. Lastly, even though *T. pusillum* is capable of producing seeds through selfing, insect pollination

produces significantly more seeds and *Meligethes* beetles were documented as floral visitors and potential pollinators. Future studies can provide further explanations for these initial findings and can assist habitat managers and biologists in future efforts to conserve and protect *Trillium pusillum*.

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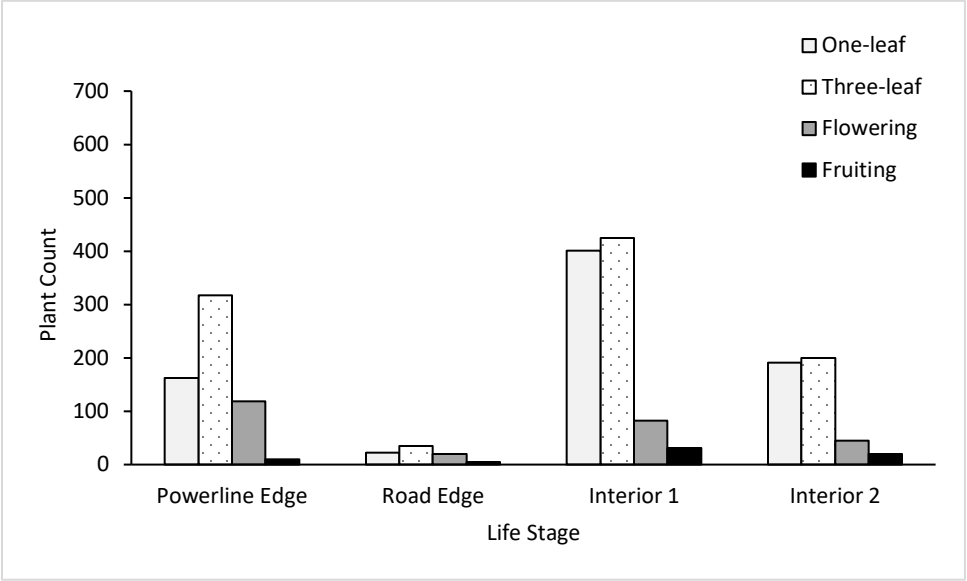
FIGURES

Figure 1: Aerial photo showing approximate locations of the Edge Effects and Life Stage Distribution transects on the study site. Photo is from Google Earth Pro version 7.1.8.3.

(a)



(b)

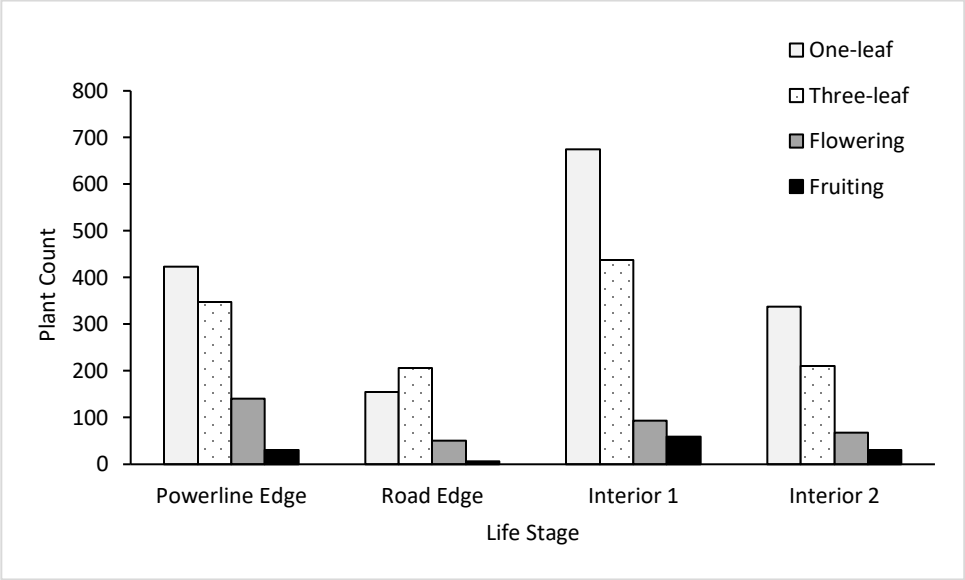
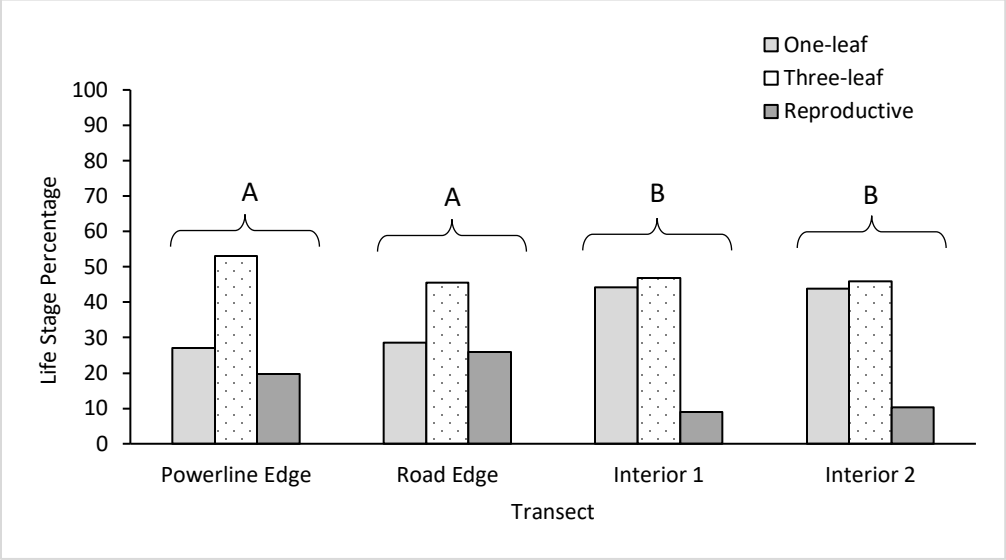


Figure 2*: Trillium pusillum* life stage counts for all four transects for both 2017 **(a)** and 2018 **(b)**. Each bar represents yearly total count of a specific life stage found in each transect.

(a)



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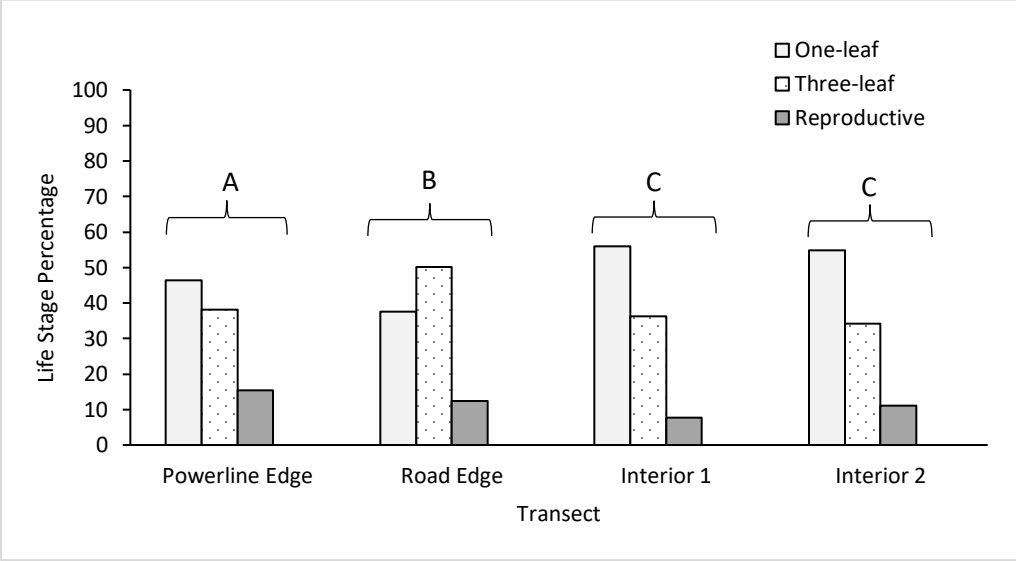
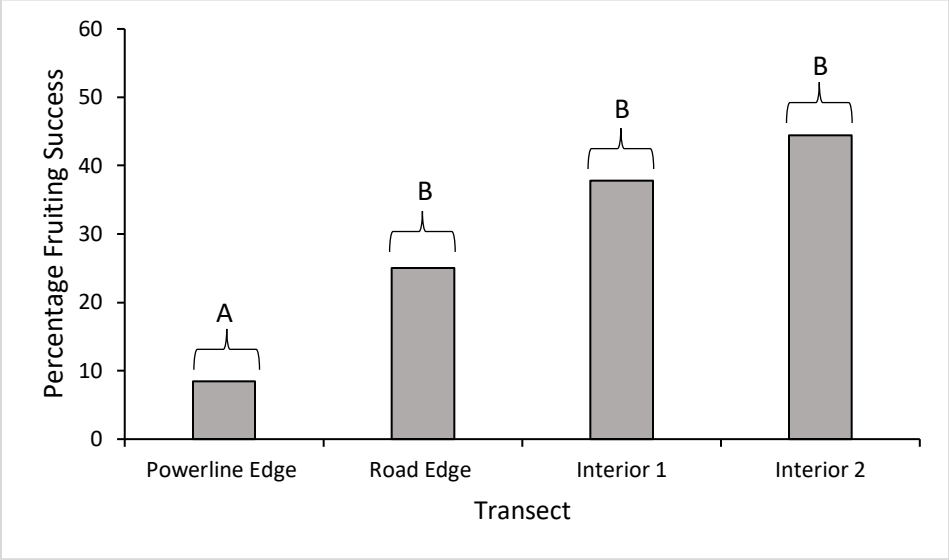


Figure 3: Comparative percentages of three life stages for all four transects in 2017 **(a)** and 2018 **(b)**. Different letters above each set of columns indicate statistically significant differences between life stage compositions of the transects (Chi-square test, $p < 0.05$)

(a)



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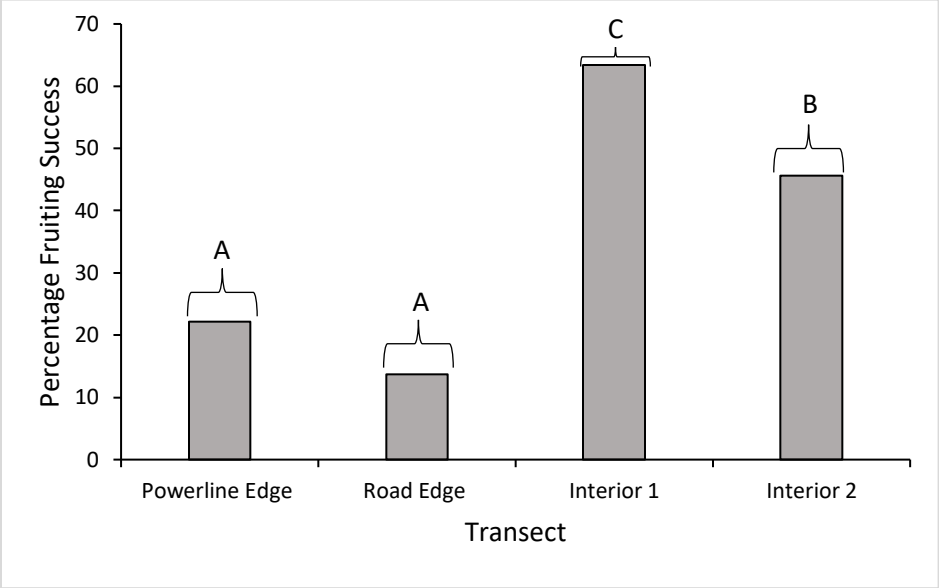


Figure 4: Fruiting success of reproductive plants for both 2017 **(a)** and 2018 **(b)**. These percentages are derived from the numbers of fruits divided by the total number of flowering plants in Figure 2. Different letters indicate statistically significant differences in fruiting success for plants on the transects (Chi-square test, $p < 0.05$)

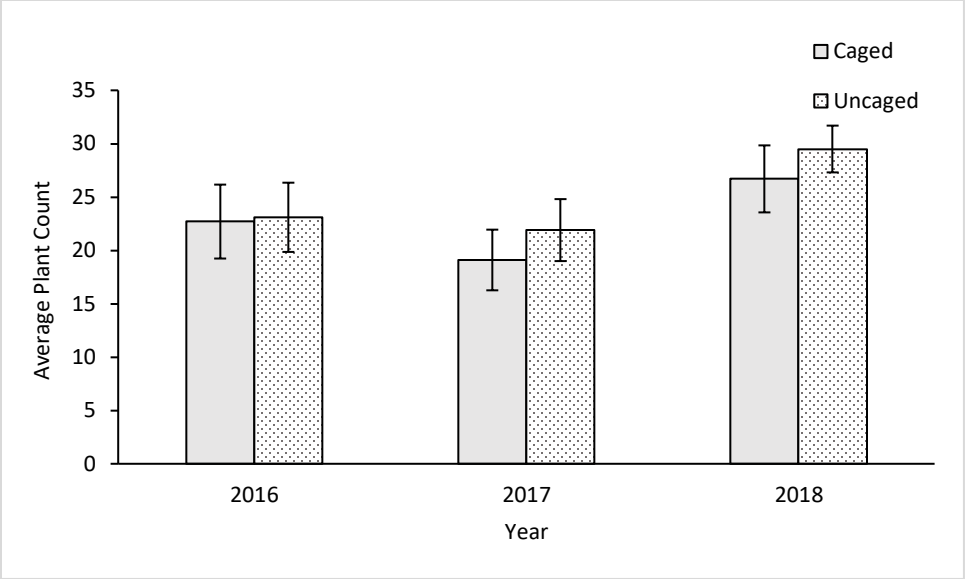
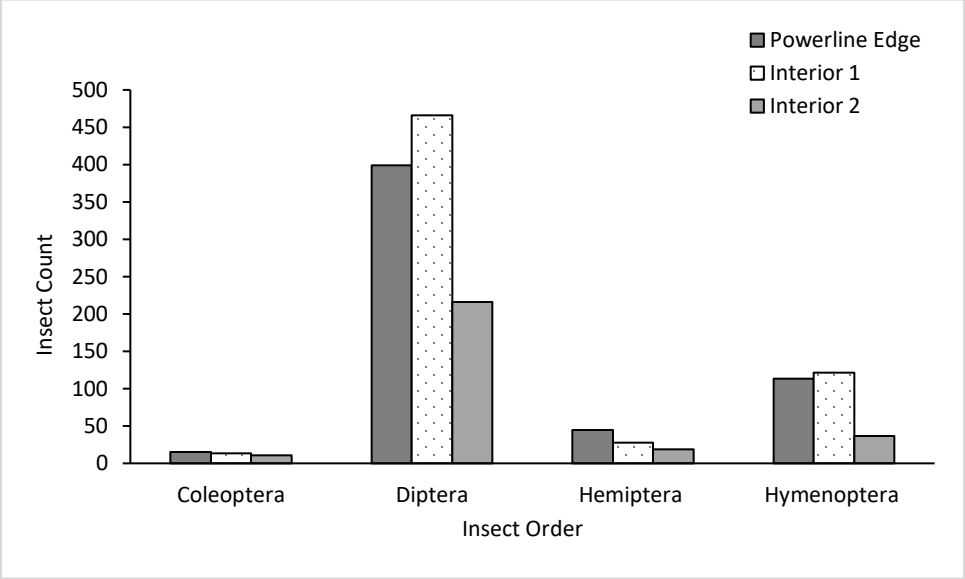


Figure 5: Mean total plant counts for caged and uncaged plots in each of the three study years. Error bars represent standard error of the mean.

(a)



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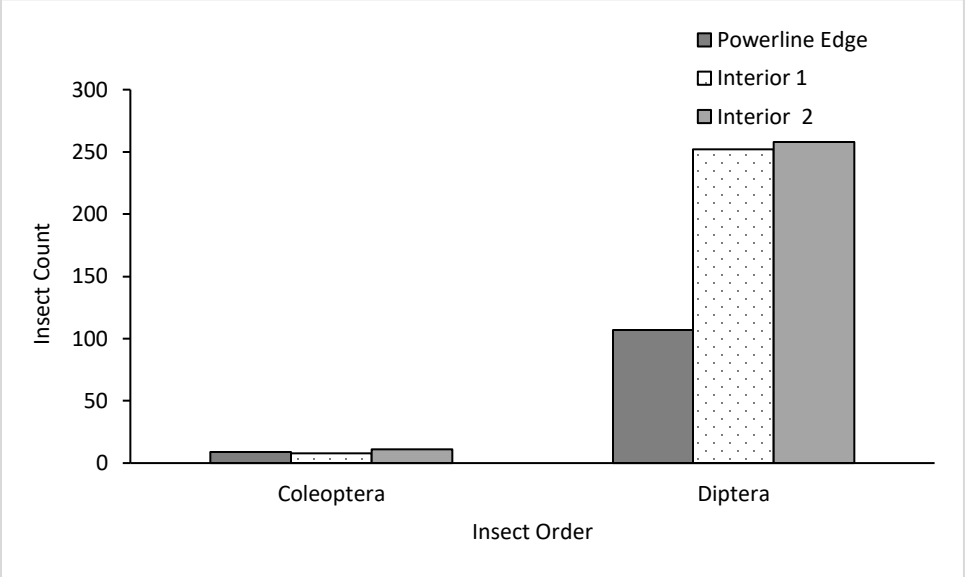

Figure 6: Counts of the most common insect orders caught in the three principal transects for both pan traps **(a)** and fermentation traps **(b)**. Total numbers of insects in each order are shown for the 2018 data collection season. Pan traps were placed on March 17, March 24, and April 2 while fermentation traps were placed on March 17 and 24. Samples were collected 24 hours after traps were placed.

TABLES

Table 1: Pairwise Chi-square test results for comparisons of life stage compositions of all pairwise combinations of transects. Chi-square values and p-values are presented for 2017 **(a)** and 2018 **(b).** There is one degree of freedom for every pairwise Chi-square test.

(a)

	Powerline Edge	Road Edge	Interior 1	Interior 2
Road Edge	2.1 ($p = 0.35$)		23.7 ($p < 0.001$)	
Interior 1	62 ($p < 0.001$)			0.58 ($p = 0.658$)
Interior 2	37.4 ($p < 0.001$)	16.3 (< 0.001)		

(b)

	Powerline Edge	Road Edge	Interior 1	Interior 2
Road Edge	16.6 ($p < 0.001$)		42.6 ($p < 0.001$)	
Interior 1	37.2 ($p < 0.001$)			5.8 ($p = 0.06$)
Interior 2	11.8 ($p = 0.002$)	31.3 ($p < 0.001$)		

Table 2: Pairwise Chi-square test results for comparisons of fruiting success of flowering plants in all pairwise combinations of transects. Chi-square values and p-values are presented for 2017 **(a)** and 2018 **(b).** There was one degree of freedom for every pairwise Chi-square test.

(a)

	Powerline Edge	Road Edge	Interior 1	Interior 2
Road Edge	4.8 ($p = 0.028$)		1.15 ($p = 0.28$)	
Interior 1	25.5 ($p < 0.001$)			0.53 ($p = 0.46$)
Interior 2	28.1 ($p < 0.001$)	2.2 ($p = 0.14$)		

(b)

	Powerline Edge	Road Edge	Interior 1	Interior 2
Road Edge	1.67 ($p = 0.2$)		32.8 ($p < 0.001$)	
Interior 1	40.2 ($p < 0.001$)			5.08 ($p = 0.02$)
Interior 2	12.0 ($p < 0.001$)	13.6 ($p < 0.001$)		

Table 3: The most common insect orders captured are summarized from both fermentation traps **(a)** and pan traps **(b).** The three taxa with the highest number of specimens are recorded for each order along with the counts for each family. The category "Other" includes all other, less numerous, families found within each order. Frequency is the percentage of all traps that contained at least one member of that particular family over the entire trapping season. Pan traps were set out on March 17, March 24, and April 2, in 2018. Fermentation traps were set out on March 17 and 24, in 2018. Samples were collected from both types of traps 24 hours after they were set up on site.

(a)

Insect Order	**Family (Taxon)**	**Insect Count**	**Frequency (%)**
Coleoptera	*Buprestidae*	4	6
	Carabidae	5	7
	Nitidulidae	7	13
	Other	21	
Diptera	*Chironomidae*	439	31
	Dolichopodidae	117	24
	Sciaridae	73	24
	Other	452	
Hemiptera	*Aphidae*	15	11
	Berytidae	4	12
	Cicadellidae	64	22
	Other	9	
Hymenoptera	*Diapriidae*	60	9
	Ichneumonidae	57	11
	Microhymenoptera	93	22
	Other	76	

(b)

Insect Order	**Family (Taxon)**	**Insect Count**	**Frequency (%)**
Coleoptera	*Nitidulidae*	26	73
	Curculionidae	1	9.1
	Staphylinidae	1	9.1
Diptera	*Drosophilidae*	563	100
	Heleomyzidae	11	55
	Sciaridae	32	73
	Other	11	