IMPACTS OF HABITAT MANAGEMENT ON TRANSLOCATION OF THE RARE TERRESTRIAL HERB *PLATANTHERA INTEGRILABIA*

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ABSTRACT

Translocation efforts are becoming widely used as a means to bolster rare species numbers in situ. *Platanthera integrilabia* is a rare terrestrial orchid endemic to the southeastern United States, and limited research has been done to investigate suitable habitat for this species. We conducted a translocation study to determine the overall success of using tubers for translocation and to investigate how light and herbivory impact translocation success. Growth measures were taken monthly during the 2018 and 2019 growing seasons, and light response measures were taken at the beginning of flowering season in both years. Our findings suggest translocation of *P. integrilabia* tubers yields initial success, especially under dense canopy cover and with limited herbivore access, but low overall rates of flowering and survival could prevent long term establishment. This study highlights the need for a longer-term, comprehensive approach to determine *P. integrilabia* resource requirements.

DEDICATION

To my Grandma, Nancy Collins, the first and greatest scientist I have ever met. Maybe the next project will have test tubes for you to wash.

ACKNOWLEDGEMENTS

First, I would like to thank my advisor Dr. Jennifer Boyd for bringing me on as a graduate student and providing an excellent example of what it means to be a scientist. I would also like to thank Dr. Hope Klug for serving on my committee and providing statistical advice. Special thank you to you both for all you have done for and endured as women in STEM. Thank you also to Geoff Call for serving on my committee, providing extensive help in the field, and providing appropriate literature and expert species information. Thanks to Adam Dattilo, who also shouldered a significant load of field work, generously shared his field notes, and provided feedback on this manuscript. Thanks to James Douglas and other members of the Tennessee Wildlife Resource Agency staff for also taking on substantial shares of field work. Thank you to my labmate, sounding board, and great friend Jared Odell, whose answer was always "yes" and to Holly Odell, giver of advice, positive thoughts, and bananas…. You two are family. And finally – but foremost – thank you to my parents for their unquestioning and multifaceted support of my academic and professional goals…. I am so very grateful for everything you both have done to get me here.

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LIST OF ABBREVIATIONS

A, photosynthesis

A_{max}, photosynthetic capacity

ANOVA, analysis of variance

LCP, light compensation point

LSD, least significant difference

PAR, photosynthetically active radiation

R_d, dark respiration

QY, quantum yield

USFWS, United States Fish and Wildlife Service

CHAPTER I

IMPACTS OF HABITAT MANAGEMENT ON TRANSLOCATION OF THE RARE TERRESTRIAL HERB *PLATANTHERA INTEGRILABIA*

1.1 Introduction

Much recent attention has been given to the role of rare species in global biodiversity loss given their disproportionate contribution to the ongoing extinction crisis (Van Calster et al. 2008, Mouillet et al. 2013). Against this backdrop, the protection and restoration of rare species has become a primary focus of conservation biology (Westin et al. 2018). Successful conservation of rare species includes understanding factors that influence and could increase their rarity and implementing actions to minimize those factors. Rare plant species often are characterized by greater habitat specialization than species that are more common (Farnsworth 2007), and the protection of critical habitat for rare species of concern is an especially important aspect of conservation that requires understanding suitable abiotic and biotic habitat (Greenwalt and Gehringer 1975, Prendergast et al. 1999, Sunil and Stohlgren 2013, Caperta et al. 2014). Such knowledge could allow for protection of viable populations in currently suitable habitat, management of habitat to continue to meet species' requirements, and restoration of degraded habitat to support populations at risk. For imperiled rare species, translocation is increasingly being used to supplement existing occurrence and establish new occurrences in suitable locations (Godefroid et al. 2016) and understanding the habitat requirements of rare species can be critical to the success of translocation efforts (Armstrong and Seddon 2008; Godefroid et al. 2011).

The orchid family (*Orchidaceae*) is experiencing exceptionally high rates of extinction (Rasmussen 1995, Cribbs et al 2003) with both more rare species (Chase 2015) and a higher proportion of threatened genera (Swarts & Dixon 2009) than any other plant family. Habitat specificity is common among orchids and, as a consequence, orchids are generally vulnerable to many forms of habitat disturbance (Tremblay et al. 1998, Bergman et al. 2006. Kolanowska et al. 2017, Fay 2018, Kottawa-Arachchi & Gunasekara 2020). The majority of orchid species are epiphytes and lithophytes, but about 25% are terrestrial (Arditti 1992), a life form that may have especially high risk of extinction (Swarts & Dixon 2009). The southeastern United States has been long recognized for its disproportionately high number of rare endemic plant species (Delcourt & Delcourt 1991, Noss et al. 2014) with areas of exceptional species uniqueness found within the southern Appalachian Mountains, across the southeastern coastal plain, and in the panhandle of Florida. The highest diversity of the more than 200 North American orchid species are found in the southeastern U.S., and the majority of these are terrestrial orchids. More than half of North American orchids are imperiled in at least a portion of their range, and every state in the United States has at least one threatened orchid species (Whigham 2012).

Platanthera integrilabia (Correll) Luer (synonym *Blephariglottis integrilabia*; white fringeless orchid) is a rare terrestrial orchid endemic to the southeastern United States that is protected under the federal Endangered Species Act as a ‘threatened’ species (USFWS 2016). Since the 1940s, *P. integrilabia* has experienced significant decline, and it is now relegated to ~60 known extant occurrences across five states (Alabama, Georgia, Kentucky, South Carolina, and Tennessee). This distribution represents a fraction of its historical range (Correll 1978, Shea 1992, USFWS 2015), which included ~90 total occurrences including some in North Carolina and possibly Virginia (Bentley 2000; USFWS 2015). Most extant occurrences of *P. integrilabia* are small (<50 flowering plants; USFWS 2015); however, some include more than 1000 individuals (NatureServe 2015). To supplement existing small and/or declining occurrences of this species and/or to potentially establish new occurrences in suitable habitat in the southeastern United States, recent efforts have been underway involving off-site propagation and field translocation (BGCI 2016).

Historically, *P. integrilabia* has been associated primarily with acidic swamps dominated by *Acer rubrum* (red maple) and *Nyssa sylvatica* (black gum) and associated with a groundcover of *Sphagnum* spp. (sphagnum moss; Bentley 2000; USFWS 2015). Underlying soils are generally sandy and lack peat accumulation despite the presence of *Sphagnum* spp. (USFWS 2015). Classic descriptions of *P. integrilabia* habitat described it as classically shaded (Luer 1975; Zettler and Fairey 1990), and related management concerns focused on the potential negative effects of common disturbances that could alter light and soil moisture availability, such as logging and pond construction (USFWS 2015). However, 11 extant *P. integrilabia* occurrences are located in electrical powerline corridor rights-of-way, which differ significantly in light intensity from its historically described habitat (USFWS 2015; Boyd et al. 2016). Anecdotal evidence from species monitoring efforts in these and other sites has suggested that opening the canopy in traditionally shaded *P. integrilabia* habitat may increase its growth and reproduction (NatureServe 2015; USFWS 2015), and limited empirical research has suggested that the species is potentially capable of physiologically adapting or acclimating to contrasting light regimes (Boyd et al. 2016).

Although the habitat requirements of *P. integrilabia* may not be as specific as historically reported, which could complicate assessments of its habitat suitability and plans for habitat management, habitat loss due to anthropogenic activities including development and silvicultural practices has been cited as a primary threat to this species (USFWS 2015). Biotic interactions including white-tailed deer (*Odocoileus virginianus*) herbivory, feral hog (*Sus scrof*a) activity (Zettler 1994, USFWS 2015), encroachment and competition from invasive nonnative plant species (Zettler & Fairey 1990), pollination inconsistencies (Zettler et al. 1996), and mycorrhizal dependence (Rasmussen & Whigham 1993, Currah et al. 1997, Zettler & McInnis 1997) also are considered to be significant concerns (USFWS 2015). The success of efforts to establish new populations of rare species depends on knowing not only their habitat requirements but also how concurrent biotic interactions – especially negative interactions with herbivores or natural enemies – that are likely in restoration sites could influence those species (Albrecht & Long 2018). Toward improving understanding of how both abiotic conditions (and their management) and biotic interactions could impact *P. integrilabia* and inform site selection and management for translocation efforts, we conducted a field translocation experiment with propagated tubers. Our research focused specifically on investigating the main effects and interactions of woody vegetation thinning (associated with changes in light availability) and white-tailed deer herbivory as likely management practices and disturbances in a protected wildlife management area in east-central Tennessee, USA.

1.2 Methodology

1.2.1 Species description

The genus *Platanthera* (Richard), commonly known as bog orchids, fringed orchids or rein orchids, contains approximately 200 species worldwide and 32 species in North America and is primarily north temperate with a few species native to tropical habitats (Sheviak, 2020). This group of orchids is terrestrial, perennial, erect to somewhat decumbent, and often succulent and identified by broad anthers. Flowers of species in this genus range in color from orange, purple, green, yellow or white with a fringed or entire labellum. *Platanthera integrilabia* is a perennial herb, which grows from a single tuber and typically emerges as a single-leaved juvenile with resulting two to three basal leaves upon flowering (NatureServe 2015). This species is characterized by generally low rates of flowering but in the flowering stage can produce a stem up to 60 cm with inflorescences of 6-20 showy white flowers clustered in loose racemes (USFWS 2015, NatureServe 2016). As suggested by its common name, the flower of *P. integrilabia* lacks fringe along the labellum and has a noticeable elongated spur extending from the back (Figure 1.1).

Figure 1.1 Occurrence of the terrestrial orchid *Platanthera integrilabia* in an acidic swamp in Tennessee, USA (left) and an inflorescence of the white fringeless flowers that distinguish this species from others in the genus (right)

1.2.2. Study location

Our study was conducted as part of the planned establishment of a new *P. integrilabia* occurrence in the Bridgestone-Firestone Centennial Wilderness, a ~4,000-hectare wildlife management area located ~90 km north of Chattanooga in White County, Tennessee, USA

(Figure 1.2). Initially, a general location for our study was determined in partnership with the Tennessee Department of Environment and Conservation, which developed a predictive habitat model based on topographic and vegetation data to identify candidate sites for the establishment of new occurrences of this species (T. Crabtree, TDEC, pers. comm.). We then surveyed viable locations to identify area in which *P. integrilabia* individuals were absent but presence of species commonly associated with *P. integrilabia*, as determined by field surveys, and the willingness of Tennessee Wildlife Resources Agency managers to manipulate light levels in the site via woody vegetation removal. White-tailed deer are common in the BFCW (J. Douglas, TWRA, Personal Communication); thus, most locations in the BFCW would afford the opportunity to investigate the potential impacts of deer herbivory on *P. integrilabia*. Our resultant study site was comprised of a 3-ha naturally forested wetland located in a streamhead swale associated with an unnamed tributary to the Caney Fork River. Vegetation in the site closely matched the species composition of the Appalachian Forested Acidic Seep association (CEGL007443) [NatureServe 2] of the Central Interior-Appalachian Seepage Swamp group (G044) [NatureServe 3] described by NatureServe.

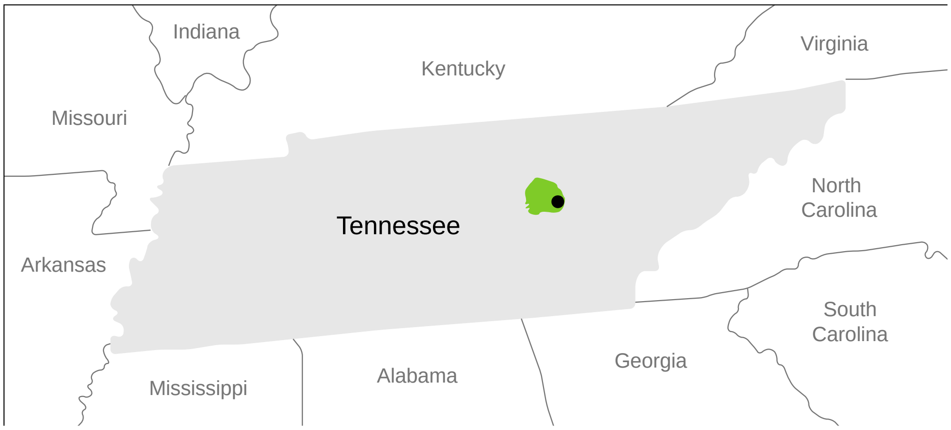


Figure 1.2 Map showing the location of the Bridgestone-Firestone Centennial Wilderness (black dot) in White County (green), Tennessee, USA

1.2.3. Experimental design

In late fall 2017, three levels of a mechanical thinning treatment designed to impact understory light availability were imposed in the study location: 1) unthinned control, 2) removal of all understory woody biomass ≥ 7.5-cm diameter at breast height (dbh; ‘moderate’ thinning), and 3) removal of all understory woody biomass ≥ 7.5-cm dbh and half of the overstory trees (‘heavy’ thinning). Each thinning treatment level was implemented using hand tools in one of three ~0.04-ha main plots designated within the study location. The influence of thinning treatment levels on canopy cover in each main plot was assessed during the peak flowering season of *P. integrilabia* in five randomly selected sampling points with a spherical crown convex densiometer (Forestry Suppliers, Inc., Jackson, Mississippi, USA). The influence of thinning on light availability to ground vegetation in each study site was assessed by measuring photosynthetically active radiation (PAR) during peak season at five randomly selected sampling points in each site on warm, clear days with a portable quantum sensor (LI-250A, LI-COR Biosciences, Lincoln, NE).

In late winter 2017, herbivory exclosures were installed in subplots within each of the main plots to provide four levels of vertebrate herbivore access to ground vegetation: 1) exclosures to protect plants from white-tailed deer only (i.e., ‘deer only’ exclusion, 2) exclosures to protect plants from white-tailed deer and smaller vertebrate grazers commonly seen in forests of our region such as rabbits and turtles (‘all herbivore’ exclusion), 3) exclosure frames to allow all grazers to access plants but account for exclosure presence (‘frames only’), and 4) non-exclosed ‘control’ subplots. Exclosures designed to protect plants from all grazers consisted of 1-m^3 frames constructed of 0.5-in-diameter (~1.25 cm) PVC that were wrapped in 1-in-aperture

(~2.5 cm) hex wire mesh (i.e., chicken wire) secured to the frames with plastic cable ties. This exclosure design was based on methods described by Benson & Boyd (2014) and Sikkema & Boyd (2015), as adapted from Frankland & Nelson (2003). Exclosures designed to protect plants from white-tailed deer only were similar but with the addition of two approximately 15-cm-wide square holes cut in two opposing sides of the wire mesh to permit smaller grazers to access plants (see Fletcher et al. 2001, Benson & Boyd 2014, Sikkema & Boyd 2015). Exclosures designed to test for the presence of exclosures but without herbivory exclusion consisted of only the PVC frames without mesh. Within each of the three thinning main plots, we placed three exclosures of each type and three 1-m^2 non-exclosed control plots in random locations with presence of sphagnum moss for a total of 12 herbivore access subplots per main plot.

Orchids can be difficult to grow from seed due to their extremely small seed size and lack of endosperm, which necessitates often-specialized relationships with mycorrhizal fungi (Zettler and McInnis 1992). Previous research on terrestrial orchids has suggested that field establishment is more successful from translocated tubers than seeds (Batty et al. 2006). As such, we utilized tubers as a means of translocating *P. integrilabia* individuals in our study sites. In early spring 2018, we obtained 72 tubers for translocation into our study sites from plants that had overwintered in controlled-environmental growth chambers following a non-destructive experiment in growth chambers located at the University of Tennessee at Chattanooga (USA) during summer and early fall 2017. The plants used in the growth chamber experiment had been grown from tubers obtained from the Atlanta Botanical Garden (Atlanta, Georgia, USA) in January 2017, which originated from plants rescued in September 2016 from a Tennessee Valley Authority powerline corridor right-of-way in Van Buren, County, Tennessee, USA that was planned for retirement and thus would lose its protection under the ESA. The 72 tubers were sorted into several size classes and then randomly and evenly divided and planted among our thinning and herbivory treatment levels in early spring 2018 such that two tubers were planted in each exclosure or non-exclosed subplot in all three thinning main plots. Within exclosures and non-exclosed subplots, the two tubers were planted ~0.5 meters apart in an east-west orientation to mitigate disproportionate shading. Because the subsequent emergence of individuals planted in 2018 was observably low during that spring and summer, we added a new cohort of 144 tubers to our study in 2019. Specifically, in early spring 2019, we obtained 144 additional tubers from the Atlanta Botanical Garden that were produced by plants rescued from the retiring TVA powerline corridor in Van Buren County, Tennessee, USA in September 2018. These 144 tubers were randomly and evenly divided and planted among our thinning and herbivory treatment levels in early spring 2019 such that four tubers were planted in each exclosure or non-exclosed subplot in all three thinning main plots. To spatially distinguish the 2018 and 2019 cohorts in subplots, tubers from the 2019 cohort were planted in the northwest, northeast, southeast, and southwest quadrats of each subplot positioned about 35 cm diagonally from the subplot corners.

1.2.4. Data collection

Subplots were monitored monthly for emerged individuals throughout the 2018 and 2019 growing seasons (i.e., May-October). Emerged individuals were categorically assessed for survival, stem production, bud or flower production, and herbivore damage. Damage by vertebrate herbivores was determined by the presence of severed leaves, petioles, and/or stems.

Concurrent with monthly counts and categorizations of individuals, we assessed growth by counting the number of leaves of all emerged individuals and estimating leaf areas with the formula for an oval $a \times b \times \pi$ in which a is the major radius equal to half of the leaf length and b

is the minor radius equal to half of the leaf width. Total basal area of emerged individuals was calculated as the sum of its leaf areas. During the latter half of each growing season, we also measured stem height of each individual as applicable. Maximum values of growth measures were determined from these monthly measurements and averaged within subplots.

To investigate potential physiological causes and consequences of our observed growth metrics, we made instantaneous gas-exchange measurements at the beginning of the flowering season in September 2018 and 2019 for all emerged *P. integrilabia* individuals. Specifically, we evaluated the photosynthetic response to light (i.e., an *A*/PAR curve) of the sole or largest basal leaf of each individual with a portable gas-exchange analyzer (LI-6400XT, LI-COR, Inc., Lincoln, Nebraska, USA) equipped with a red/blue LED light source (LI-COR 6400-02B) and CO_2-mixing system on warm, clear late mornings and early afternoons. The steady-state responses of photosynthesis to externally supplied PAR provided in 12 steps from 2000 to 0 μmol photons m^{-2} s^{-1} (i.e., full sunlight to total darkness) were measured. At each PAR setpoint, a photosynthetic measurement was made after gas exchange had equilibrated, which was determined when the coefficient of variation for the CO_2 partial pressure differential between the sample and reference analyzers was below 1% with a minimum wait time of 90 s. During photosynthetic light-response measurements, leaf temperature was kept at 22°C (the mean average daily temperature for September across sites) using thermoelectric coolers.

1.2.5. Data analyses

To examine the influence of vegetation thinning and vertebrate herbivory on *P. integrilabia* growth, maximum values of growth measures were determined from monthly measurements of the number of leaves, total leaf area, stem height, and number of buds/flowers per individual of each cohort. The photosynthetic light-response curve generated for each measured *P. integrilabia* individual was analyzed by fitting the data to a modified non-rectangular hyperbola model (Ye et al. 2013) with the Microsoft Excel Solver tool (Microsoft Corporation, Redmond, Washington, USA) to estimate the maximum rate of light-saturated photosynthesis (A_{max}; μmol CO_2 m^{-2} s^{-1}), rate of dark respiration (R_d; μmol CO_2 m^{-2} s^{-1}), apparent quantum yield (QY; A_{max}; μmol CO_2 μmol^{-1} photons) as a measure of the efficiency with which light is converted into fixed carbon, and light compensation point (LCP; μmol photons m^{-2} s^{-1}) as a measure of the minimal light required for photosynthetic carbon gain to offset respiratory carbon loss.

Due to differences in propagule sourcing, the 2018 and 2019 cohorts were analyzed as distinct groups in our statistical analyses; as such, direct comparisons among years cannot be made from our data. Within each cohort, chi-square goodness-of-fit tests were conducted to determine if the proportions of emerged plants, plants producing stems, plants producing buds or flowers, and plant exhibiting damage from vertebrate herbivores differed among the vegetation thinning and herbivory exclosure treatment levels as main factors. In the event of a significant main effect, comparisons among various levels of a treatment were made with subsequent pairwise chi-square tests. Within each cohort, we used a two-way mixed-model analysis of variance (ANOVA) to test for the main effects and interactions of vegetation thinning and herbivory as fixed factors on growth measures and physiological variables derived from photosynthetic light-response measures. Following a significant ANOVA, differences between treatment levels were combined determined with least significant difference (LSD) post hoc analysis. Results of all statistical tests were considered significant if $p < 0.05$. All statistical analyses were performed in SPSS (SPSS Statistics Version 26 software, IBM Corp., Armonk, New York, USA).

1.3 Results

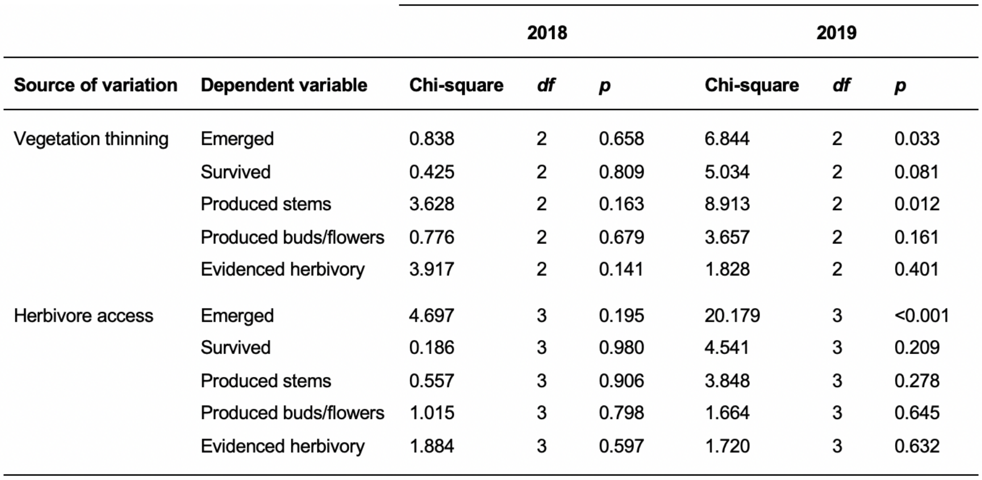
Across main plots, there were significant differences in peak-season canopy cover ($F_{2,12}$=11.490, p=0.002) and light intensity ($F_{2,12}$=7.391, p=0.008). Overall, vegetation thinning had a negative influence on canopy cover and a positive influence on light availability. Percent canopy cover was 91.7 ± 2.6 (mean ± SE), 78.4 ± 2.0, and 55.1 ± 8.9 in the unthinned, moderately thinned, and heavily thinned main plots, respectively. Hemispherical photographs taken from the center of each main plot during peak season depict these differences in canopy cover (Figure 1.3). The corresponding light availability of the unthinned, moderately thinned, and heavily thinned main plots was 236.9 ± 74.1, 601.7 ± 138.9, and 923.1 ± 151.9 μmol photons m^{-2} s^{-1}, respectively.

Figure 1.3 Hemispherical photographs taken from the center of unthinned (left), moderately thinned (center), and heavily thinned (right) main plots utilized in our study site in the Bridgestone-Firestone Centennial Wilderness, Tennessee, USA during the peak flowering season of *Platanthera integrilabia*

Across vegetation thinning and herbivore access treatment levels, approximately 59% of tubers produced emerged plants in the 2018 cohort and 70% of tubers produced emerged plants in the 2019 cohort. In 2018, the proportion of emerged plants did not differ across vegetation thinning or herbivore access treatment levels (Table 1.1). In contrast, the proportion of successful emergence from tubers translocated in 2019 differed across both vegetation thinning and herbivore access treatment levels (Table 1.1) with both thinning and herbivore access negatively impacting emergence. Specifically, the proportion of total tubers that emerged from the 2019 cohort was about 46% greater in the unthinned main plot than in the heavily thinned main plot (χ^2=6.385, *df* =1, *p*=0.012). Across vegetation thinning treatment levels, the proportion of tubers that emerged in non-exclosed subplots was about 47% less than emergence when all herbivores were excluded (χ^2=14.831, *df* =1, *p*<0.001) and 43.3% less than emergence when white-tailed deer only were excluded (χ^2=10.812, *df* =1, *p*=0.001).

Of total emerged plants in 2018 and 2019, approximately 56% and 23% survived through the growing season to the time of peak flowering, respectively. However, survival did not differ across vegetation thinning or herbivory access treatments in either year (Table 1.1). In the 2018 cohort, about 19% and 12% of plants that emerged produced stems and buds or flowers, respectively, while about 9% and 2% of plants that emerged from the 2019 cohort produced stems and buds or flowers. The proportion of emerged plants in the 2018 cohort that produced stems and buds or flowers did not differ across vegetation thinning or herbivore access treatment levels (Table 1.1). Although the proportion of emerged plants in the 2019 cohort that produced buds or flowers similarly was not influenced by vegetation thinning or herbivore access, the proportion of emerged plants that produced stems in the unthinned main plot main plot was 87.7% less than in the heavily thinned main plot (χ^2=6.355, *df* =1, *p*=0.012) in 2019. In addition, the proportion of emerged plants that produced stems in the moderately thinned main plot was nearly 65% less than that in the heavily thinned plot (χ^2=4.454, *df* =1, *p*=0.035) in 2019. Damage by grazing herbivores was evident on about 12% of emerged plants in the 2018 cohort and 28% of emerged plants in the 2019 cohort, but the proportion of plants evidencing damage by herbivores did not differ across vegetation thinning or herbivore access treatments during either year.

Table 1.1 Results of chi-square analyses to test the influence of vegetation thinning and vertebrate herbivore access on the numbers of *Platanthera integrilabia* individuals that emerged from tubers planted in early 2018 and 2019 in a forested study site in the Bridgestone-Firestone Centennial Wilderness, Tennessee, USA and the number of emerged plants that survived, produced stems, produced buds or flowers, and evidenced herbivory during the subsequent growing season



		2018			2019		
Source of variation	**Dependent variable**	**Chi-square**	***df***	***p***	**Chi-square**	***df***	***p***
Vegetation thinning	Emerged	0.838	2	0.658	6.844	2	0.033
	Survived	0.425	2	0.809	5.034	2	0.081
	Produced stems	3.628	2	0.163	8.913	2	0.012
	Produced buds/flowers	0.776	2	0.679	3.657	2	0.161
	Evidenced herbivory	3.917	2	0.141	1.828	2	0.401
Herbivore access	Emerged	4.697	3	0.195	20.179	3	<0.001
	Survived	0.186	3	0.980	4.541	3	0.209
	Produced stems	0.557	3	0.906	3.848	3	0.278
	Produced buds/flowers	1.015	3	0.798	1.664	3	0.645
	Evidenced herbivory	1.884	3	0.597	1.720	3	0.632

The maximum total leaf area of emerged *P. integrilabia* individuals in the 2018 cohort was not influenced significantly by the main effects of vegetation thinning ($F_{2,32}$=0.040, p=0.961), herbivore access ($F_{3,31}$=2.127, p=0.124), or the interaction of these treatments ($F_{6,28}$=0.462, p=0.829). However, the maximum total leaf area of individuals in the 2019 cohort was influenced significantly by the main effects of vegetation thinning ($F_{2,32}$=9.276, p=0.001), herbivore access ($F_{3,31}$=4.499, p=0.013), and their interactions ($F_{6,28}$=2.824, p=0.033). When vegetation was moderately thinned, the total leaf area of *P. integrilabia* individuals was more than double than when vegetation was heavily thinned (96.0 ± 12.4 vs. 43.4 ± 6.1 cm^2, p=0.004) and more than 50% greater than when vegetation was not thinned (66.2 ± 7.6 cm^2, p=0.049), but total leaf area did not differ significantly between the unthinned and heavily thinned treatment levels. Across vegetation thinning treatment levels, maximum total leaf area of *P. integrilabia* individuals in non-exclosed 'control' subplots (42.0 ± 6.6 cm^2) was about 54% less than that of plants protected from all vertebrate herbivores (91.4 ± 13.6 cm^2, p=0.002) and 48.2% than that of plants protected from deer only (81.1 ± 14.3 cm^2, p=0.014). Individuals inside frames without chicken wire (i.e. 'frames only') also had significantly lower maximum total leaf area (53.1 ± 21.0 cm^2) than plants protected from all vertebrate herbivores (p=0.018), but the total leaf area of plants inside 'frames only' did not differ from plants in non-exclosed 'control' subplots or those protected from deer only. The interaction of vegetation thinning and herbivore access indicate that the influence of herbivore access on total leaf area was mostly significant when vegetation was unthinned or moderately thinned compared to when vegetation was heavily thinned (Figure 1.4). In contrast to total leaf area, the maximum stem height of plants that produced stems was not influenced significantly by the main effects of vegetation thinning or herbivore access or their interactions in either the 2018 cohort (vegetation thinning, $F_{2,5}$=0.704, p=0.644; herbivore access, $F_{3,4}$=0.021, p=0.994; vegetation thinning × herbivore access, $F_{6,1}$=0.320, p=0.672) or the 2019 cohort (vegetation thinning, $F_{2,6}$=7.514, p=0.068; herbivore access, $F_{3,5}$=2.414, p=0.237; vegetation thinning × herbivore access, $F_{6,2}$=0.048, p=0.841).

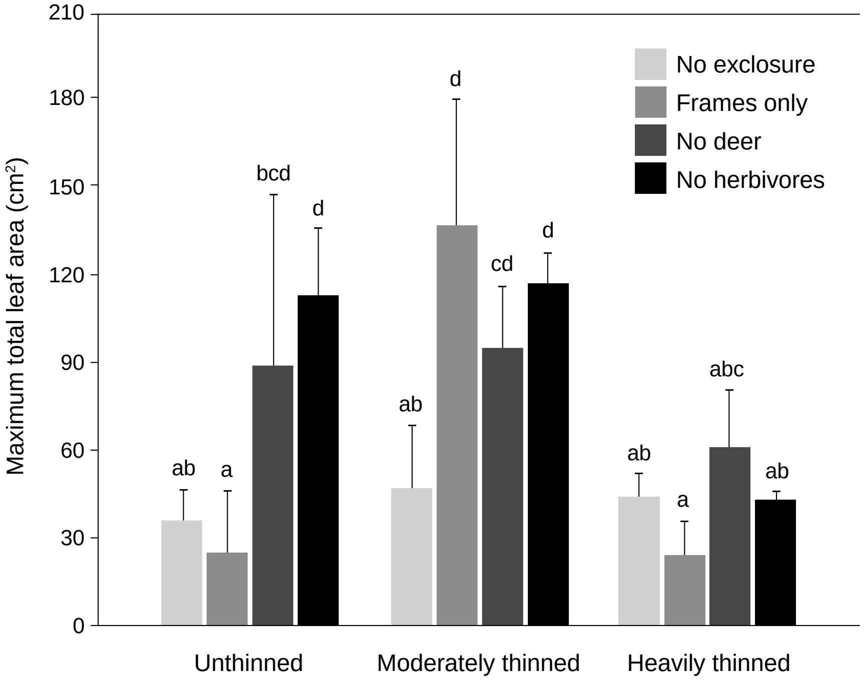
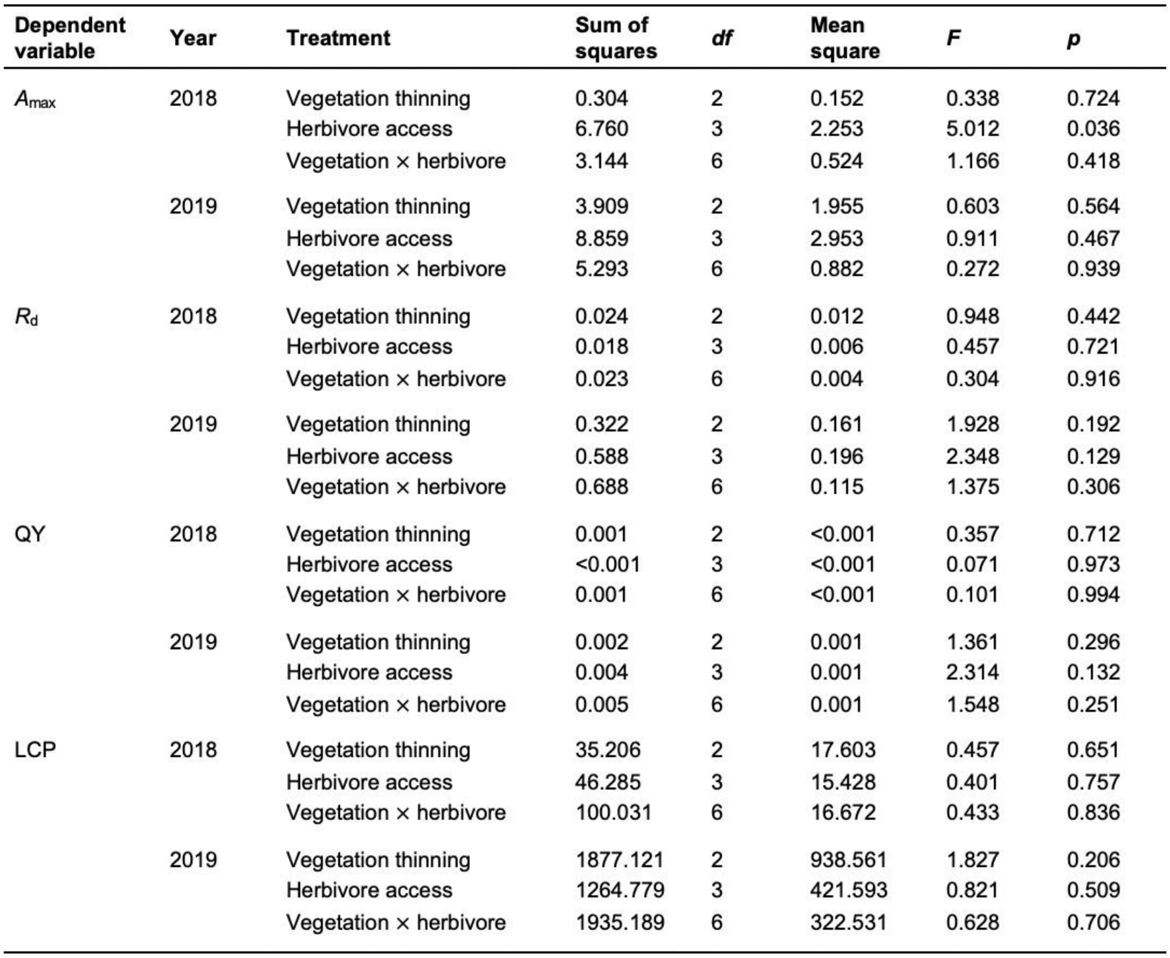


Figure 1.4 Mean maximum total leaf area (cm^2) exhibited by *Platanthera integrilabia* individuals emerged from tubers planted in early 2019 during the subsequent growing season as determined from monthly measurements in a forested study site in the Bridgestone-Firestone Centennial Wilderness, Tennessee, USA. Tubers were planted and plants were grown in main plots with unthinned, moderately thinned, and heavily thinned woody vegetation in vertebrate herbivore access subplots that included unexclosed controls (no exclosure), open exclosure frames (frames only), exclosures designed to exclude white-tailed deer only (no deer), and exclosures designed to exclude all vertebrate grazers (no herbivores). Error bars represent ± 1 SE of the mean. Values shown below the same letter are not significantly different at the $p \leq 0.05$ level of significance

Differences in leaf-level physiology as assessed during the peak flowering season of *P. integrilabia* were limited to a significant main effect herbivore access on leaf A_{max} (Table 1.2), which did not exhibit any trend associated with degree of herbivore access or exclusion. Specifically, leaf A_{max} of plants protected from all vertebrate herbivores (2.83 ± 0.49 µmol CO_2 m^{-2} s^{-1}) was more than double that of plants surrounded by open frames (1.29 ± 0.28 µmol CO_2 m^{-2} s^{-1}; $p = 0.011$) and about 75% greater than that of plants protected from white-tailed deer only (1.61 ± 0.18 µmol CO_2 m^{-2} s^{-1}; $p = 0.030$). The leaf A_{max} of plants in non-exclosed subplots

(2.09 ± 0.26μmol CO_2 m^{-2} s^{-1}) did not differ from that of plants in any of the other herbivore access treatment levels.

Table 1.2. Results of two-way analysis of variance (ANOVA) to test for the main effects and interactions of vegetation thinning and vertebrate herbivore access on the leaf-level physiology of *Platanthera integrilabia* individuals that emerged from tubers planted in early 2018 and 2019 in a forested study site in the Bridgestone-Firestone Centennial Wilderness, Tennessee, USA. All variables were derived from photosynthetic light-response curves; variables include maximum rate of light-saturated photosynthesis (A_{max}; μmol CO_2 m^{-2} s^{-1}), rate of dark respiration (R_d; μmol CO_2 m^{-2} s^{-1}), apparent quantum yield (QY; μmol CO_2 $μmol^{-1}$ photons), and light compensation point (LCP; μmol photons m^{-2} s^{-1})



Dependent variable	Year	Treatment	Sum of squares	*df*	Mean square	*F*	*p*
A_{max}	2018	Vegetation thinning	0.304	2	0.152	0.338	0.724
		Herbivore access	6.760	3	2.253	5.012	0.036
		Vegetation × herbivore	3.144	6	0.524	1.166	0.418
	2019	Vegetation thinning	3.909	2	1.955	0.603	0.564
		Herbivore access	8.859	3	2.953	0.911	0.467
		Vegetation × herbivore	5.293	6	0.882	0.272	0.939
R_d	2018	Vegetation thinning	0.024	2	0.012	0.948	0.442
		Herbivore access	0.018	3	0.006	0.457	0.721
		Vegetation × herbivore	0.023	6	0.004	0.304	0.916
	2019	Vegetation thinning	0.322	2	0.161	1.928	0.192
		Herbivore access	0.588	3	0.196	2.348	0.129
		Vegetation × herbivore	0.688	6	0.115	1.375	0.306
QY	2018	Vegetation thinning	0.001	2	<0.001	0.357	0.712
		Herbivore access	<0.001	3	<0.001	0.071	0.973
		Vegetation × herbivore	0.001	6	<0.001	0.101	0.994
	2019	Vegetation thinning	0.002	2	0.001	1.361	0.296
		Herbivore access	0.004	3	0.001	2.314	0.132
		Vegetation × herbivore	0.005	6	0.001	1.548	0.251
LCP	2018	Vegetation thinning	35.206	2	17.603	0.457	0.651
		Herbivore access	46.285	3	15.428	0.401	0.757
		Vegetation × herbivore	100.031	6	16.672	0.433	0.836
	2019	Vegetation thinning	1877.121	2	938.561	1.827	0.206
		Herbivore access	1264.779	3	421.593	0.821	0.509
		Vegetation × herbivore	1935.189	6	322.531	0.628	0.706

1.4 Discussion

1.4.1. Overall translocation success

The translocation of tubers to the field has been used previously in attempt to establish new occurrences of terrestrial orchid species of concern (Batty et al. 2006). Although limited, the results of such attempts focused on rare terrestrial orchids of Australia suggest that establishment from tubers is high relative to *in situ* sowing of seed and actively growing seedlings; however, differences in the success of establishment across orchid taxa warrants investigations that are genera and/or species specific (Batty et al. 2006). To our knowledge, our investigation comprises the first assessment of the viability of tuber translocation as a means of establishing new occurrences of *P. integrilabia* or any of its rare congeners. Previous research that investigated the establishment of *P. integrilabia* seedlings germinated from seed in field-collected soil in a controlled glasshouse environment reported that 46% of seedlings successfully established to the point of producing the single basal leaf characteristic of non-flowering *P. integrilabia* (Zettler and McInnis 1992). Relative to these previous results, the establishment of translocated *P. integrilabia* tubers in our study site across vegetation thinning and herbivory access treatment levels was more successful with more than half of tubers emerging into vegetative plants in both the 2018 and 2019 cohorts. When considered in isolation, our results suggest that tuber translocation may be a preferred method for the establishment of new *P. integrilabia* occurrences, as well as potentially the supplementation of existing occurrences.

Beyond emergence and initial establishment of vegetative plants, however, the successful establishment of new viable (i.e., self-sustaining) occurrences of *P. integrilabia* will require survival and growth of translocated individuals to reproductive maturity and eventually successful reproduction for recruitment. The survival of translocated terrestrial orchids in the field has varied across taxa and methodologies – including propagule type and field conditions – but with low survival rates being common (Batty et al. 2006, Smith et al. 2009). For example, research comparing the 1-year survival of tubers of three terrestrial orchid species native to Western Australia planted in the field reported survival of around 50% for all three species but with survival dropping to 0-30% by the third year depending on species (Batty et al. 2006); however, a fourth species exhibited an 80% five-year survival rate from mature tubers. Although the 1-year survival rate of the emerged *P. integrilabia* individuals in our 2018 cohort was also around 50%, our 2019 cohort exhibited half of this rate and survival rates for both cohorts would be substantially less if expressed relative to the numbers of tubers planted rather than emerged plants. Although limited, previous research has reported extremely low flowering rates of terrestrial orchid individuals established from translocated tubers of several species, suggesting that the low proportion of flowering plants in both our 2018 and 2019 cohorts may be typical for first-year tuber-propagated plants (Smith et al. 2009). In contrast, reported flowering rates when orchid seedlings were used for translocation have been substantially greater, although these rates varied across specific seedling propagation methods (Wright et al. 2009).

1.4.2. Influence of habitat management on establishment and fitness

The establishment, survival, growth, and reproduction of terrestrial orchids can be influenced by a wide range of abiotic and biotic factors that merit consideration in translocation efforts (Brundrett 2007). Among these factors, shade has been associated with decreased survival and reproduction (Brundrett 2007). While the largest known natural occurrence of *P. integrilabia* occurs in a shaded site and previous published research on *P. integrilabia* has suggested that it may be able to physiologically tolerate a variety of light environments (Boyd et al. 2016), management reports describe that occurrences have responded positively to canopy clearing and negatively to succession of woody vegetation (USFWS 2016, Williams 2000, Littlefield). Observations also suggest that *P. integrilabia* may require a certain critical level of light to flower (Richards 2016), which could explain such positive responses. Although vegetation thinning did not influence flowering of *P. integrilabia* individuals emerging from translocated tubers during our study, the increased number of individuals in our 2019 cohort producing stems – a process associated with flowering in this species – when vegetation was heavily thinned suggests that its reproduction may be enhanced by light availability. We suggest that any negative impacts of shade on individual fitness of *P. integrilabia* could be exacerbated at the occurrence-level by inbreeding depression, which can threaten the viability of small populations (Frankham 2005, O'Grady et al. 2006) Although some congeneric species have been reported to have facultative outcrossing breeding systems (Gregg 1990), which could minimize the negative impacts of inbreeding (Wilson 1983); pollinator observations of *P. integrilabia* have concluded that outcrossing is likely limited in this species in all but its largest occurrence (Zettler and McInnis 1996).

In other terrestrial orchid species, it has been proposed that plants must reach a threshold size in terms of leaf area before flowering can occur (Willems 1989) and that the impacts of shading on photosynthesis at a fundamental level could underlie observable declines in performance (Willems 2000). If a critical plant size is similarly required for reproduction to occur in *P. integrialbia*, our total leaf area findings could indicate that moderate thinning may benefit this species by encouraging early growth. While light did not influence photosynthesis in the present study, our previous research comparing the performance of natural *P. integrilabia* occurrences in contrasting light environments revealed that individuals in open occurrences had significantly greater photosynthetic rates than individuals in shadier sites (Boyd et al. 2016). However, we also found that individuals growing in contrasting light microsites within occurrences (such as those caused by canopy gaps) did not exhibit different photosynthetic capacities, suggesting that photosynthetic differences among natural *P. integrilabia* occurrences result from long-term adaptions to site conditions rather than short-term acclimation (Boyd et al. 2016).

Herbivory can negatively influence plant survival, growth, and reproduction (Maron and Crone 2006, Moreira et al. 2019), and grazing by invertebrates has been recognized as a major cause of mortality in terrestrial orchids (Brundrett 2007). Grazing by white-tailed deer in has been cited as a specific threat to *P. integrilabia*. and floral grazing, in particular has been observed in numerous *P. integrilabia* occurrences (Shea 1992, USFWS 2016, Zettler and Fairley 1990). Although observed herbivore damage in both our 2018 and 2019 cohorts was minimal and low overall flowering rates may have limited our ability to detect any grazer preference for flowers or flowering plants, our findings do suggest that white-tailed deer may negatively impact the emergence of *P. integrilabia* individuals from translocated tubers. Specifically, we noted that emergence in our 2019 cohort was significantly greater when tubers were protected from white-tailed deer. In addition, protecting plants from all vertebrate herbivores did not result in greater emergence rates than protecting plants from deer only, suggesting that deer may be a primary cause of low emergence. Because an analysis of white-tailed deer diets in forests of the southeastern United States revealed that deer feed primarily on aboveground biomass and fruits regardless of season (Johnson et al. 1995), we suggest that deer may have grazed young *P. integrilabia* in our study site soon after emergence rather than uprooting tubers. Alternatively, it is possible that feral hogs (*Sus scrof*a), which also have been cited as potentially detrimental to *P. integrilabia* (USFWS 2016) could have uprooted tubers prior to emergence; however, we did not observe any signs of such activity in our site. Our results also suggest that white-tailed deer can negatively impact the aboveground growth of young *P. integrilabia* individuals following emergence (see Figure 1.4) but that vegetation thinning could minimize these impacts, perhaps by deterring deer from feeding in more open locations and/or allowing plants to prioritize growth over herbivory defense strategies in response to grazing.

1.4.3. Recommendations for Platanthera integrilabia *conservation and management*

Rare plant species often are characterized by greater habitat specialization than species that are more common (Farnsworth 2006), and the assessment of habitat suitability is fundamental to species conservation efforts (Captera et al. 2004). The results of our research suggest that translocation of tubers as a means to establish new populations and/or supplement existing populations of *P. integrilabia* could result in successful early establishment of individuals but that low rates of survival and flowering are possible and should be considered in translocation plans. Our findings indicate that management of woody vegetation in *P. integrilabia* occurrences could have different effects on individuals that are life stage dependent. Specifically, we found the emergence was greatest in when woody vegetation was left intact, aboveground growth (i.e., total leaf area) was greatest with moderate vegetation thinning, and that reproduction could be enhanced by heavy thinning. We also found that increased light availability could benefit *P. integrilabia* in areas where white-tailed deer are abundant by moderating the impacts of grazing.

Based on our findings, we suggest that *P. integrilabia* occurrences, whether newly established or natural, be monitored for demographic information that could be used to guide vegetation management plan and that plans may need to be adjusted if demography changes. For example, declining *P. integrilabia* numbers have been attributed to in part to low flowering rates in many occurrences and management of such occurrences could prioritize approaches to help elicit flowering. But it also has been suggested that bolstered reproductive success after canopy thinning may last only a few seasons and that the sizes of occurrences eventually begin to decline with the encroachment of other understory species (Shea 1992). A managed approach of canopy thinning to promote flowering followed by subsequent understory thinning a few years later to promote vegetative growth might be effective in generating and sustaining substantial reproduction rates and occurrence sizes. Deer fencing installed in some *P. integrilabia* occurrences has been associated with reducing grazing of vegetative and flowering plants (USFWS 2016), and higher rates of herbivory at sites in which fencing has been damaged emphasize the potential effectiveness of fencing in areas with abundant deer populations (USFWS 2016).

1.4.4. Additional considerations and future research

This report describes the first attempt to establish a new occurrence of rare and federally threatened *P. integrilabia* in the field via tuber translocation. Overall, our results suggest that both high light availability associated with heavy clearing of woody vegetation and vertebrate herbivory could negatively impact translocation success via reduced emergence and subsequent growth. Although flowering as a measure of reproductive effort was not similarly influenced by the studied environmental factors, we concede that low sample sizes due to emergence and survival rates and/or inherently low first-year flowering rates of translocated tubers as evidenced for other terrestrial orchid species (Smith et al. 2009) could have limited our ability to detect such environmental effects. Further longer-term investigations of the responses of different life stages of *P. integrilabia* – whether in extant or newly established occurrences – to light availability and vertebrate herbivory as factors implicated in its decline (USFWS 2016) could help to evaluate these proposed implications and inform conservation efforts.

Other biotic factors known to influence terrestrial orchid populations also could contribute to the success of *P. integrilabia* translocation efforts in critical ways. Because appropriate symbiotic mycorrhizal fungi and pollinator species are necessary for the completion of orchid life cycles (McCormick and Jacquemyn 2014), and thus ultimately fitness, consideration of such interactions is important to the success of orchid translocations (Wright et al. 2009, Brundrett 2007). Previous work has demonstrated the importance of appropriate fungal symbionts to *P. integrilabia* survival and establishment (Zettler and McInnis 1992) and cross-pollination by day-flying Lepidopteran species observed in the largest known natural occurrence of *P. integrilabia* (in McMinn County, Tennessee, USA) was associated with relatively high rates of fruit set (Zettler and McInnis 1992). We used a habitat suitability model based on associated species to inform study sites for our translocation project, but notably did not evaluate soil fungal communities or pollinator activity in our research. We suggest that seed baiting (Rasmussen and Whigham 1993, Brundrett et al. 2003) and pollinator baiting (Phillips et al. 2009) and/or observations could be valuable tools for refining the selection of suitable *P. integrilabia* translocation sites in the future by detecting the presence or absence of these important associates.

In addition to more comprehensive site assessments, the success of terrestrial orchid translocation also could be improved by further considerations of the propagules to be used. Generally, the use of larger propagules and propagules derived from larger founder populations in rare species translocation efforts has resulted in greater success of those efforts (Godefroid et al. 2011, Albrecht and Maschinski 2012) For terrestrial orchids, the findings of previous research suggest that larger tubers experienced increased survival and flowering rates relative to smaller tubers (Smith et al. 2009), likely due to the critical role of tubers as storage reserves (Batty et al. 2006). In our research, we standardized tuber sizes across treatment levels in attempt to isolate the influence of vegetation thinning and vertebrate herbivory on *P. integrilabia* emergence, survival, growth, and reproduction and thus did not account for the potential influence of tuber size on translocation success. Tracking initial tuber sizes in future projects could help to elucidate the influence of tuber size on establishment and guide the future use of tubers for *P. integrilabia* translocation. Investigations of seed germination and glasshouse establishment of *P. integrilabia* have indicated that seed sourced from larger populations is more viable than seed sourced from smaller populations, suggesting that genetic inbreeding in smaller population could be associated with decreased fitness (Zettler and McInnis 1992). Although we worked with propagules sourced from a single relatively small occurrence of *P. integrilabia* in need of timely rescue for our translocation study, future translocation projects that include tubers from multiple occurrences of different sizes could reveal similar fitness patterns. However, combining tubers from multiple source populations should be guided by knowledge of the population genetic structure of this species, which is lacking. Because terrestrial orchid tubers can remain dormant for several years (Kery and Gregg 2004), we also suggest that longer-term evaluations of translocation efforts involving tubers may be warranted to more comprehensively assess their success. Such evaluations could be conducted in conjunction with existing annual monitoring efforts for extant *P.integrilabia* occurrences across its range.

1.4.5. Conclusions

The rapid pace of contemporary environmental change due to anthropogenic activities and influences (Palumbi 2001) has been implicated as a particular threat to rare species (Mouillet et al. 2013). For *P. integrilabia*, such threats include the destruction and modifications of its habitat due to development, silviculture, incompatible right-of way management, succession, and overabundant herbivore populations, among other factors (USFWS 2016). The use of translocation as a potentially useful conservation strategy for imperiled species such as *P. integrilabia* in the face of rapid environmental change has become globally widespread (Silcock et al. 2019). When translocation efforts are combined with research designed to assess the influence of factors such as site conditions and/or propagule types and sources on outcomes, the success of translocation efforts could be improved and the management of new and extant occurrences could be informed. Yet even when successful, translocation should be considered an enhancement to species and habitat conservation efforts rather than a band-aid approach to activities that threaten the persistence of rare species in natural occurrences. We also caution that translocation efforts could negatively affect the viability of source populations; as such, translocation as a conservation strategy may be most appropriate for the rescue of populations that are otherwise likely to be extirpated.

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