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Impacts of Forest Thinning and White-Tailed Deer Herbivory on Translocation of the Rare Terrestrial Orchid *Platanthera integrilabia*

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Abstract: Translocation is increasingly being used to supplement existing occurrences and establish new occurrences of rare plant species, but translocation success is dependent on understanding responses to habitat conditions and management. *Platanthera integrilabia* (white fringeless orchid) is a rare terrestrial orchid species presently found in mostly small occurrences that comprise a fraction of its historical distribution and abundance in the southeastern United States. We investigated the influence of shade and white-tailed deer herbivory, as cited concerns for this species, on the early success of its translocation from tubers as determined through measures of emergence, survival, growth, and reproduction of two cohorts. Our findings suggest that translocation from tubers could be a viable option to assist the conservation of *P. integrilabia* relative to its propagation from seed, but that low early emergence, survival, and flowering rates should be considered in translocation plans. Our results also indicate that translocation and ongoing habitat management should consider the potential for light availability to differentially impact distinct plant life stages and influence deer herbivory. We recommend that additional translocation studies designed to investigate the influence of site conditions on outcomes could improve the success of such efforts as well as inform the management of extant occurrences.

Keywords: light availability; protected species; species conservation; white fringeless orchid

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 [1,2]. Against this backdrop, the protection and restoration of rare species has become a primary focus of conservation biology [3]. Successful conservation and restoration of rare species includes understanding factors that influence and could increase their rarity and implementing actions to minimize those factors. Rare plant species are often characterized by greater habitat specialization than species that are more common [4] and the protection of critical habitats for rare species of concern is an especially important aspect of conservation that requires understanding the characteristics of suitable abiotic and biotic habitats [5–8]. Such knowledge could allow for protection of viable populations in currently suitable habitats,

management of habitats to continue to meet species' requirements, and restoration of degraded habitats to support populations at risk. For imperiled rare species, translocation is increasingly being used to supplement existing occurrences and establish new occurrences in suitable locations [9] and understanding the habitat requirements of rare species can be critical to the success of translocation efforts [10,11]. The Orchidaceous family is experiencing exceptionally high rates of extinction [12,13] with both rarer species [14] and a higher proportion of threatened genera [15] 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 [16–20]. The majority of orchid species are epiphytes and lithophytes, but about 25% are terrestrial [21], a life form that may have especially high risk of extinction [15]. The southeastern United States has been long recognized for its disproportionately high number of rare endemic plant species [22,23] 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 [24].

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 [25]. 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 [26–28], which included ~90 total occurrences including some in North Carolina and possibly Virginia [28,29]. Most extant occurrences of *P. integrilabia* are small (<50 flowering plants) [28]; however, some include more than 1000 individuals [30]. 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 [31].

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) [28,29]. Underlying soils are generally sandy and lack peat accumulation despite the presence of *Sphagnum* spp. [28]. Classic descriptions of *P. integrilabia* habitat described it as classically shaded [32,33], 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 [28]. 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 [28,34]. Anecdotal evidence from species monitoring efforts in these and other sites has suggested that opening the canopy in traditionally shaded *P. integrilabia* habitats may increase its growth and reproduction [28,30], and limited empirical research has suggested that the species is potentially capable of physiologically adapting or acclimating to contrasting light regimes [34].

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 [28]. Biotic interactions including white-tailed deer (*Odocoileus virginianus*) herbivory, feral hog (*Sus scrofa*) activity [28,35], encroachment and competition from invasive nonnative plant species [35], pollination inconsistencies [36], and mycorrhizal dependence [37–39] also are considered to be significant concerns [28]. 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 [40]. 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.

2. Materials and Methods

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 [41]. 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 [30]. 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 [28,30]. 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).



Figure 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).

2.2. Study Location

Our study was conducted during the 2018 and 2019 growing seasons as part of the planned introduction of a new *P. integrilabia* occurrence in the Bridgestone–Firestone Centennial Wilderness (BFCW), a ~4000 hectare wildlife management area located primarily on the Cumberland Plateau ~90 km north of Chattanooga, TN, USA (Figure 2). Initially, a general location for the introduction 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 throughout its current range [42]. We then performed field surveys to ground truth the predictive model with the goal of identifying areas in which *P. integrilabia* was absent but species commonly associated with *P. integrilabia* were present, and where Tennessee Wildlife Resources Agency managers could manipulate light levels via

woody vegetation removal. White-tailed deer are common in the BFCW [43]; 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) [44] of the Central Interior Appalachian Seepage Swamp group (G044) [45] described by NatureServe.

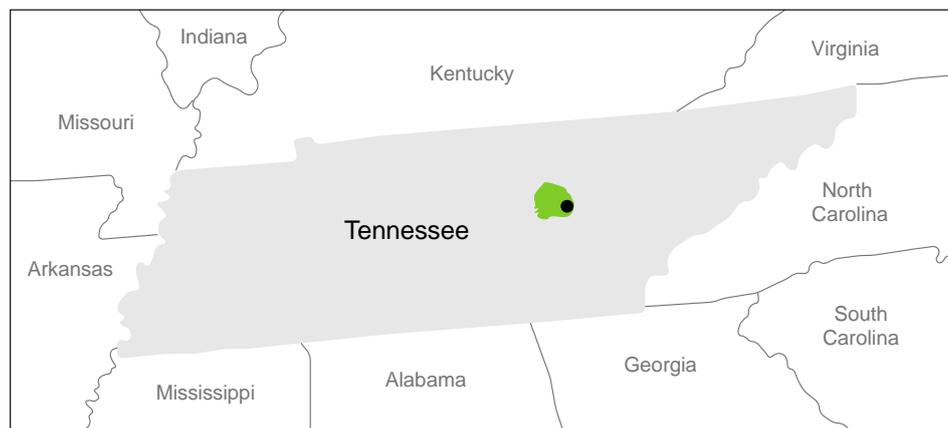


Figure 2. Map showing the location of the Bridgestone–Firestone Centennial Wilderness (black dot) in White County (green), TN, USA.

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 (i.e., shaded by taller canopy vegetation) woody stems ≥ 7.5 -cm diameter at breast height (dbh; “moderate” thinning), and (3) removal of all non-canopy woody stems and approximately 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. To prevent resprouting of woody stems, all cut stems were treated with an appropriate herbicide (Pathway Herbicide RTU, Corteva Agriscience, Wilmington, DE, USA). 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, MS, 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, USA).

In late winter 2018, 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 m³ frames constructed of 0.5 in diameter (~ 1.25 cm) polyvinyl chloride (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 previous methods used in studies focused on white-tailed deer herbivory in regional forest understory sites [46–48]. 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 [46,47,49]). Enclosures designed to test for the presence of exclusions but without herbivory exclusion consisted of only the PVC frames without mesh. Within each of the three thinning main plots, we placed three enclosures of each type and three 1 m² non-excluded 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 [39]. Previous research on terrestrial orchids has suggested that field establishment is more successful from translocated tubers than seeds [50]. As such, we utilized tubers as a means of translocating *P. integrilabia* individuals in our study sites. Here, we use the term “translocation”, as it was historically defined by the International Union of Conservation of Nature (IUCN) as any technique used to introduce, reintroduce, or reinforce in situ plant populations by introducing additional individuals from outside the occurrence as an applied management technique and conservation approach [51].

In March 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 (ABG; Atlanta, GA, 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, TN, USA that was planned for retirement. When a powerline is retired and associated structures are removed, right-of-way vegetation management is typically discontinued, which would facilitate relatively rapid environmental change due to succession of relatively open habitat into dense forest. In total, 36 emerged *P. integrilabia* individuals were documented in the right-of-way. These plants and surrounding soil were removed from the site and transported to ABG in tubs where they were gently separated from the surrounding soil. During this process, >200 small tubers not associated with emerged plants were found in the soil. All tubers were transferred to an outdoor holding bed in the conservation nursery at ABG, where they were held until utilized for research. The 72 tubers obtained in March 2018 were transported to the field in their pots where they were removed and sorted into several size classes. The sorted tubers were then randomly and evenly divided and planted among our thinning and herbivory treatment levels such that two tubers were planted in each enclosure or non-excluded subplot in all three thinning main plots. Within enclosures and non-excluded subplots, the two tubers were planted ~0.5 m 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 March 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 Tennessee Valley Authority (TVA) powerline corridor in Van Buren County, TN, USA in September 2016. These 144 tubers, which were obtained as bare roots, 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 enclosure or non-excluded 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. Seasonal weather conditions as reported at the nearby Crossville Memorial Whitson Field Airport Weather Observation Stations were mostly similar between years [52]. Mean daily average temperature from April–May was 18.1 °C and 18.3 °C, and total precipitation was 49.4 cm and 45.6 cm for 2018 and 2019, respectively. From June–September, mean daily average temperatures were 22.8 °C and 23.1 °C, and total precipitation was 45.6 cm and 27.1 cm for 2018 and 2019, respectively. Differences in seasonal precipitation between years was primarily due to nearly 16 cm more precipitation recorded in September 2018 than in September 2019.

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 or 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 within a 1-week period at the beginning of the flowering season in September 2018 and 2019 for all emerged *P. integrilabia* individuals in accordance with methods used in previous research of this species [34]. 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, NE, USA) equipped with a red/blue LED light source (LI-COR 6400-02B) and °C CO₂-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 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (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 CO₂ 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.

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. 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 [53] with the Microsoft Excel Solver tool (Microsoft Corporation, Redmond, WA, USA) to estimate the maximum rate of light-saturated photosynthesis (A_{max} ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), rate of dark respiration (Rd; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), apparent quantum yield (QY; $\mu\text{mol CO}_2 \mu\text{mol}^{-1} \text{ photons}$) as a measure of the efficiency with which light is converted into fixed carbon, and light compensation point (LCP; $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) as a measure of the minimal light required for photosynthetic carbon gain to offset respiratory carbon loss.

We used a one-way analysis of variance (ANOVA) to test for the influence of vegetation thinning on canopy cover and light intensity as dependent variables reflecting abiotic site conditions. 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 plants exhibiting damage from vertebrate herbivores differed among the vegetation thinning and herbivory exclusion 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 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. These data were tested for normality with the Shapiro–Wilk

test. 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, NY, USA).

3. Results

There were significant plot 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 \pm 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 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 $923.1 \pm 151.9 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively.



Figure 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, TN, USA during the peak flowering season of *Platanthera integrilabia*.

In total, 70% of tubers produced emerged plants in the 2019 cohort and 59% of tubers produced emerged plants in the 2018 cohort. Successful emergence in the 2019 cohort was negatively impacted both by thinning and herbivore access (Table 1). 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 ($\chi^2 = 6.385$, $df = 1$, $p = 0.012$) and about 47% and 43% less in non-excluded subplots than when all herbivores were excluded ($\chi^2 = 14.831$, $df = 1$, $p < 0.001$) and when white-tailed deer only were excluded ($\chi^2 = 10.812$, $df = 1$, $p = 0.001$), respectively. In contrast, the proportion of emerged 2018 plants did not differ across vegetation thinning or herbivore access treatment levels (Table 1). Of the total emerged plants in 2019 and 2018, approximately 23% and 56% 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).

In the 2019 cohort, approximately 9% and 2% of emerged plants produced stems and buds or flowers, respectively, while about 19% of the emerged plants in the 2018 cohort produced stems and 12% produced flowers or buds. The proportion of emerged 2019 plants that produced stems was 88% less in the unthinned main plot and 65% less in the moderately thinned main plot ($\chi^2 = 4.454$, $df = 1$, $p = 0.035$) than in the heavily thinned main plot ($\chi^2 = 6.355$, $df = 1$, $p = 0.012$). In contrast, the proportion of emerged plants in the 2018 cohort that produced stems did not differ across vegetation thinning or herbivore access treatment levels (Table 1). Similarly, budding/flowering was not influenced by vegetation thinning or herbivore access in either the 2019 or 2018 cohort (Table 1).

Table 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, TN, USA and the number of emerged plants that survived, produced stems, produced buds or flowers, and evidenced herbivory during the subsequent growing season.

Source of Variation	Dependent Variable	2018			2019		
		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 size of *P. integrilabia* individuals in the 2019 cohort (as determined by maximum total leaf area) was significantly influenced by vegetation thinning ($F_{2,32} = 9.276, p = 0.001$), herbivore access ($F_{3,31} = 4.499, p = 0.013$), and the interaction of these treatments ($F_{6,28} = 2.824, p = 0.033$). Specifically, individuals were significantly larger in the moderately thinned main plot ($96.0 \pm 12.4 \text{ cm}^2$) than in the heavily thinned main plot ($43.4 \pm 6.1 \text{ cm}^2, p = 0.004$) and unthinned main plot ($66.2 \pm 7.6 \text{ cm}^2, p = 0.049$). The size of *P. integrilabia* individuals in non-exclosed “control” subplots ($42.0 \pm 6.6 \text{ cm}^2$) was less than that of plants protected from all vertebrate herbivores ($91.4 \pm 13.6 \text{ cm}^2, p = 0.002$) and plants protected from deer only ($81.1 \pm 14.3 \text{ cm}^2, p = 0.014$). Individuals inside frames without chicken wire (i.e., “frames only”) also were significantly smaller ($53.1 \pm 21.0 \text{ cm}^2$) than plants protected from all vertebrate herbivores ($p = 0.018$). The influence of herbivore access on total leaf area of plants in the 2019 cohort was mostly significant when vegetation was unthinned or moderately thinned compared to when vegetation was heavily thinned (Figure 4). In contrast, the size of individuals in the 2018 cohort was not significantly influenced by 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 ($F_{6,28} = 0.462, p = 0.829$). Similarly, the maximum stem height of plants that produced stems was not influenced by vegetation thinning, herbivore access, or the interactions of these treatments in either the 2019 cohort (vegetation thinning, $F_{2,6} = 7.514, p = 0.068$; herbivore access, $F_{3,5} = 2.414, p = 0.237$; interaction, $F_{6,2} = 0.048, p = 0.841$) or 2018 cohort (vegetation thinning, $F_{2,5} = 0.704, p = 0.644$; herbivore access, $F_{3,4} = 0.021, p = 0.994$; interaction, $F_{6,1} = 0.320, p = 0.672$).

Leaf A_{max} of *P. integrilabia* individuals from the 2019 cohort protected from all vertebrate herbivores ($2.83 \pm 0.49 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was significantly greater than that of plants surrounded by open frames ($1.29 \pm 0.28 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}; p = 0.011$) and plants protected from white-tailed deer only ($1.61 \pm 0.18 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}; p = 0.030$). However, there was no influence of vegetation thinning or the interaction of vegetation thinning and herbivore access on leaf A_{max} in this cohort (Table 2). Leaf A_{max} of individuals in the 2018 cohort was not influenced by herbivore access, vegetation thinning, or the interaction of these treatments (Table 2).

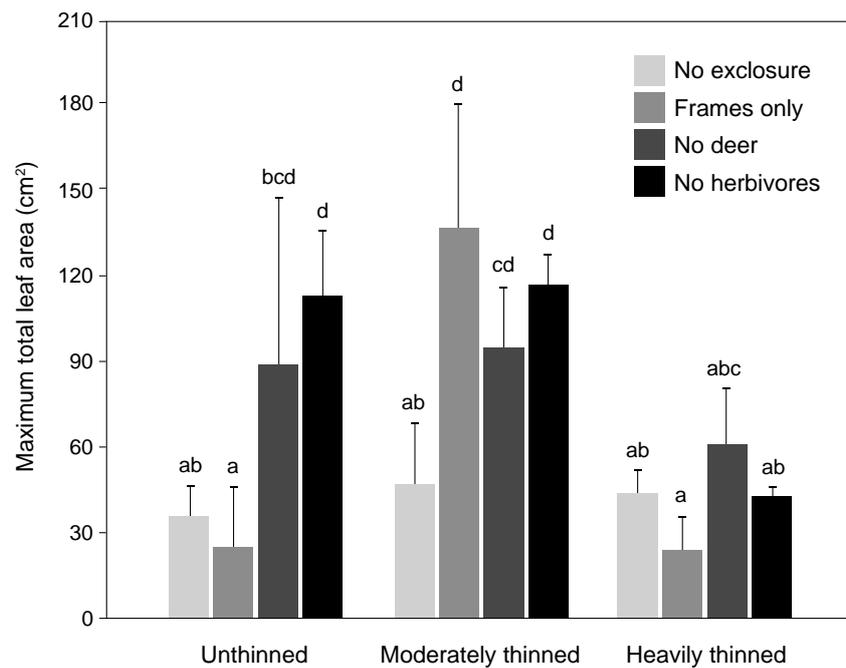


Figure 4. Mean maximum total leaf area (cm²) exhibited by *Platanthera integrilabia* individuals emerged from tubers planted in early 2019 during the subsequent growing season as determined from monthly measurements in a forested study site in the Bridgestone–Firestone Centennial Wilderness, TN, USA. 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 unenclosed controls (no enclosure), open enclosure frames (frames only), enclosures designed to exclude white-tailed deer only (no deer), and enclosures designed to exclude all vertebrate grazers (no herbivores). These subplots were considered as replicates in our experimental design; $n = 3\text{--}4$ for all means depicted. 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.

Table 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, TN, USA. All variables were derived from photosynthetic light-response curves; variables include maximum rate of light-saturated photosynthesis (A_{\max} ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), rate of dark respiration (R_d ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), apparent quantum yield (QY; $\mu\text{mol CO}_2 \mu\text{mol}^{-1} \text{ photons}$), and light compensation point (LCP; $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$).

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 \times 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 \times 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 \times 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 \times herbivore	0.688	6	0.115	1.375	0.306

Table 2. Cont.

Dependent Variable	Year	Treatment	Sum of Squares	df	Mean Square	F	p
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

4. Discussion

4.1. Overall Translocation Success

The translocation of tubers to the field has been used previously in an attempt to establish new occurrences of terrestrial orchid species of concern [50]. 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 [50]. 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* [39]. 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 2018 and 2019. When considered in isolation, our results could 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. However, differences between the greenhouse study and our field study limit the direct comparability of results. In particular, the greenhouse study utilized seedlings propagated from seed collected from multiple natural occurrences of *P. integrilabia* and noted that different occurrences were associated with different emergence rates [39]. The greenhouse study also involved the inoculation of pasteurized soil with mycorrhizal fungal isolates, most of which did not promote seedling establishment [39]. Our tubers were derived from a single *P. integrilabia* occurrence, and we did not include an inoculation treatment in our study; as such, it is possible that differences in emergence were influenced by these factors rather than by seedling source (i.e., translocated tubers vs. newly germinated seeds).

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—and through time but with low survival rates being common [50,54]. 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 [50]; however, a fourth species exhibited an 80% five-year survival rate from mature tubers. Although the survival rate of the emerged *P. integrilabia* individuals in our 2018 cohort through a first growing season 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. While we focused on the survival of plants emerged from translocated tubers through a first growing season in our study, yearly monitoring would be required to generate a longer-term prognosis of survival. 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 [54]. 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 [55].

4.2. Influence of Light and Deer Herbivory on *Platanthera integrilabia*

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 [56]. Among these factors, shade has been associated with decreased survival and reproduction [56]. While the largest known natural occurrence of *P. integrilabia* occurs in a shaded site and previously published research on *P. integrilabia* has suggested that it may be able to physiologically tolerate a variety of light environments [34], management reports describe that occurrences have responded positively to canopy clearing and negatively to succession of woody vegetation [27,57,58]. Observations also suggest that *P. integrilabia* may require a certain critical level of light to flower [59], 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. Research focused on another rare terrestrial orchid species of eastern North America reported increased stem production after significant canopy thinning followed by increased seed capsule production, further suggesting that stem growth could be a potential indicator of reproductive potential [60].

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 [61] and that the impacts of shading on photosynthesis at a fundamental level could underlie observable declines in performance [62]. If a critical plant size is similarly required for reproduction to occur in *P. integrilabia*, 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 [34]. 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 adaptations to site conditions rather than short-term acclimation [34].

Herbivory can negatively influence plant survival, growth, and reproduction [63,64], and grazing by invertebrates has been recognized as a major cause of mortality in terrestrial orchids [56]. Associations between sharp declines in orchid species, including *Platanthera spp.*, and decreased rates of deer harvesting, suggest that white-tailed deer in particular could especially influence orchid species decline and, in some cases, extirpation [65]. Grazing by white-tailed deer has been cited as a specific threat to *P. integrilabia*, and floral grazing, in particular, has been observed in numerous *P. integrilabia* occurrences [27,28,33]. 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. Our results also suggest that white-tailed deer can negatively impact the aboveground growth of young *P. integrilabia* individuals following emergence (see Figure 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.

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 [4], and the assessment of habitat suitability is fundamental to species conservation efforts [8]. The results of our research suggest that translocation of tubers as a means to establishing new populations and/or supplementing 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 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 plans and that plans may need to be adjusted if demography changes. For example, declining *P. integrilabia* numbers have been attributed in part to low flowering rates in many occurrences and management of such occurrences could prioritize approaches to help elicit flowering. However, it has also 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 [27]. 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. Although not included in our study, we suggest that prescribed burning could be explored as an alternative to mechanical thinning, given the reported fire tolerance of *P. integrilabia* [66] and other species in the genus [67,68] and the potential for appropriately timed burning to enrich soil in ways that could benefit orchids [69]. Deer fencing installed in some *P. integrilabia* occurrences has been associated with reducing grazing of vegetative and flowering plants [28], and higher rates of herbivory at sites in which fencing has been damaged emphasize the potential effectiveness of fencing to protect orchids in areas with abundant deer populations [28]. Feral hogs have also been cited as a threat to *P. integrilabia* abundance [25], and while we did not observe evidence of hog damage at our site, we suggest that protecting plants from deer with fencing could help to prevent hog damage as well.

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 [54] 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 [28] 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 [70], and thus, ultimately, fitness, consideration of such interactions is important to the success of orchid translocations [55,56]. Both specific fungal symbiont and pollinator species associated with *P. integrilabia* have been identified [36,39], and we suggest that these biotic interactions be considered in site selection for future translocation efforts. 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 [11,71]. For terrestrial orchids, the findings of previous research suggest that larger tubers experienced increased survival and flowering rates relative to smaller tubers [54], likely due to the critical role of tubers as storage reserves [50]. 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 [39]. 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 [72], 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.

5. Conclusions

The rapid pace of contemporary environmental change due to anthropogenic activities and influences [73] has been implicated as a particular threat to rare species [2]. 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 [28]. 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 [74]. 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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Conflicts of Interest: G.C. is an employee of the funding sponsor, the United States Fish and Wildlife Service, and was involved in project conceptualization and carrying out mechanical thinning treatments and outplanting of orchid tubers. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the United States Fish and Wildlife Service.

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