1 **Can putative indicator species predict habitat quality for American ginseng?**

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16 **ABSTRACT**

17 American ginseng, *Panax quinquefolius* L., is a long-lived medicinal understory herb,
18 which has been heavily harvested since the 1700's. Because of the economic value of the
19 root, and the increasing rarity of this plant, *P. quinquefolius* is often reintroduced across

its range. Land managers and hobby growers recommend using ‘associate species’ as a way to determine ideal site conditions for reintroduction. However, the accuracy of these putative indicator species in identifying sites that will maximize growth of this rare herb has not been tested. Using a long-term ecological dataset of 26 populations, we evaluated if 20 putative indicators (herbs, shrubs, and trees) could predict *P. quinquefolius* performance, as measured by the relative growth rate of the leaf area, at the population and microsite level. Of the indicators, only one tree species was able to predict positive performance. If a *P. quinquefolius* was within 10 meters of a *Liriodendron tulipifera* L., the plant would have increased growth, in terms of leaf area, as compared to plants that were not within 10 meters of this tree. Surprisingly, the presence of most putative indicator species was found to be unreliable as a site quality measure. At the population level, four putative indicators, *Aralia nudicaulis* L., *Acer rubrum* L., *Betula lenta* L., and *Lindera benzoin* (L.) Blume, were actually contra-indicators, as their presence at a site implied lower *P. quinquefolius* performance. If *Podophyllum peltatum* L. was absent from a site, but *B. lenta* present, *P. quinquefolius* had reduced growth as compared to plants present in other combinations of *P. peltatum* and *B. lenta*. The results from this study have important implications for *in situ* conservation strategies of this rare medicinal plant. Planting *P. quinquefolius* in sites that increase performance can help ensure that reintroduction projects likely have a greater chance of success, effectively reducing the waste of time, money, and resources spent on projects that have lower levels of success.

1. INTRODUCTION

The understory is responsible for 90% of the biodiversity of the eastern deciduous forest (Gilliam, 2007). These species in the forest understory are ecologically significant;

many of these species are economically and medicinally important (Burkhart and Jacobson, 2009; Chamberlain et al., 2013). Often, rare understory forbs are the most sensitive to disturbance, and their abundance is likely to decrease as disturbance events occur over time (Wiegmann and Waller, 2006). Knowing the most effective ways to implement conservation, by assessing habitat quickly and effectually for reintroduction of these sensitive species, can be vital to preserve diversity.

Analysis of indicator species within a habitat can be used to assess ecosystem health or determine habitat quality (Landres et al., 1988). This technique can be particularly useful in predicting habitat suitability for a single rare or endangered plant species (Gregory et al., 2010; Ren et al., 2010; Vittoz et al., 2006). Species' niches, in general, are multi-dimensional in ways that are difficult to quantify (Pulliam, 2000). If indicator species could be found that respond to environmental variation in space and time in a similar manner to a rare species, knowing the detailed niche requirements of the rare species would not be necessary to determine where that species might be found, or where that species could be successfully reintroduced (Ren et al., 2010).

One example where indicator species analysis could be used to support reintroduction would be the efforts to restore populations of the uncommon to rare species, American ginseng (*Panax quinquefolius* L.). *P. quinquefolius* is often reintroduced, as this species is a valuable medicinal plant that is harvested by individuals in North America and sold into the international market (Burkhart, 2013; Burkhart et al., 2012). In addition to being economically valuable, *P. quinquefolius* is ecologically important as a food source, as the wood thrush (*Hylocichla mustelina*) consume the flesh from the berries (Hruska et al., 2014), whitetail deer (*Odocoileus virginianus*) browse the

leaves (McGraw and Furedi, 2005), and rodents eat the roots (Davis and Persons, 2014).

Reintroduction, or woods grown cultivation, of *P. quinquefolius* is an *in situ* strategy for conservation (Burkhart, 2013). This process can be difficult as the niche of this species is broad, as it can be found on a variety of slopes, aspects, elevations, over a vast latitudinal and longitudinal breadth (McGraw et al., 2003, 2013), and under a wide array of overstory species (McGraw et al., 2013). The ideal indicator species would be a plant whose niche would overlap completely with the portion of this niche where *P. quinquefolius* has rapid growth rate and high reproductive success (Fig. 1a).

Experienced cultivators have already adopted the indicator species approach to make recommendations to others wanting to produce ‘wild simulated’ or ‘woods grown’ roots (Burkhart, 2013). *P. quinquefolius* habitat indicators are frequently listed in the popular literature (Pritts, 1995), in anthropological studies (Hufford, 2003), and in ecological studies (Burkhart, 2013). Examples of several putative indicator species include Jack-in-the-pulpit (*Arisaema triphyllum* (L.) Schott), rattlesnake fern (*Botrychium virginianum* (L.) Sw.), and sugar maple (*Acer saccharum* Marshall) (Anderson et al., 1993; Fountain, 1986). Previous research on indicators and *P. quinquefolius* involved visiting sites with this herb present, and cataloguing the species in the adjacent area (Anderson et al., 1993; Burkhart, 2013; Fountain, 1986). While there has been extensive work to determine these associated species, there is no research on their ability to predict sites that maximize performance.

Reintroduction can be a valuable tool in rare plant conservation (Guerrant, 2012), but there are risks in planting cultivated, woods grown, or wild simulated *P. quinquefolius* (Apsley and Carroll, 2004; Davis and Persons, 2014; Punja, 2011). Often,

because of this potential failure, individuals who are interested in agroforestry of this species are given the recommendation of introducing a few plants or seeds to test the site before starting a large scale reintroduction project (Beyfuss, 1992; Carroll and Apsley, 2004; Davis and Persons, 2014). However, as this understory herb matures slowly (McGraw et al., 2013), this time frame may be ill-suited for a species that needs conservation priority.

The use of association analysis alone to guide *P. quinquefolius*' planting could be flawed for several reasons. As an economically valuable plant that has been harvested since the 1700's (McGraw et al., 2013), plants may be found now primarily in fringe habitats (Burkhart, 2013; McGraw et al., 2003) (Fig. 1b). Due to localized extirpation, remaining populations could be 'sink populations' (Pulliam, 1988). Second, this plant's niche is large (McGraw et al., 2003), but the likelihood is low that the niches of these indicator species overlap neatly within the niche of this rare herb. Instead, the niche of an indicator species might be smaller in some dimension, and therefore, only a good indicator in a part of its range (Fig. 1c). Finally, the niche of the putative indicator could be larger, such that its presence is not a good indicator of suitable habitat everywhere it is found (Fig. 1d). The validity of indicator species in predicting quality habitat needs to be rigorously tested so time, money, effort, and plants are not wasted on unsuccessful reintroduction projects.

Understanding if the use of indicator species can signal if a site is within the optimum niche will be beneficial for future reintroduction projects. The existence of a suite of indicators for *P. quinquefolius* pre-supposes a Clementsian ecological niche, in which groups of species share a common set of environmental requirements (Clements,

1916). Given that research more generally supports the Gleasonian individualistic niche (Denslow, 2014), and communities do not respond in a unified fashion to temporal or spatial environmental gradients (Davis, 1984), the more likely scenario is that there will be different indicators for quality *P. quinquefolius* habitat in different parts of its range. Using a new methodology that investigates if the presence of indicator species indicates positive performance, while the absence indicates poor performance, will help us understand if these species are, in fact, universal indicators of prime habitat.

In this study we investigated the potential use of indicator species in a new way, we determined, based on the presence or absence of neighboring companion species, if we could predict a rare species' performance, at varying scales across its range. Our questions were: (1) Do populations of *P. quinquefolius* with putative indicator species present at the site have higher levels of performance than those populations that do not have those indicator species? (2) Do individual *P. quinquefolius* plants located near putative indicator species have higher levels of performance than plants that are not near indicator species, suggesting certain indicator species represent an ideal microsite environment?

2. METHODS

2.1. Study Species

P. quinquefolius is a representative herbaceous species of the eastern deciduous forest due to its life history traits, long-lived nature, and wide geographic range in North America (McGraw et al., 2013). Further, this plant can be found on a variety of slopes, aspects and elevations (McGraw et al., 2003). A single population of plants can range in physical size from .04 to 4 hectares. Each population is made up of smaller groups of

individuals; we refer to as “clusters.” Populations of *P. quinquefolius* often have fewer than 200 individuals, but within the population, cluster size can range from 1 plant to more than 100 plants/m² (McGraw et al., 2013; Wagner and McGraw, 2013).

2.2. Census

Twenty-six natural populations of *P. quinquefolius*, found across seven states, were used for this research during the summer of 2013. The plants included in this study were visited twice each year, to measure size and reproductive output. Each individual plant was cryptically tagged with a numbered aluminum nail. During the spring census, we measured the length and width of the longest leaflet on each leaf. These populations were censused every year since at least 2004 with the same methodology. Using this geographically and temporally extensive data set, we were able to analyze *P. quinquefolius* growth over time. Growth is a strong metric for plant performance, as the size of the plant corresponds with reproductive effort (McGraw, 2001). For each year, total plant leaf area was calculated using a previously derived allometric relationship using the length and width of the longest leaflet on each of the leaves.

$$\text{Leaf Area} = -49.66 + (6.87 * \text{Leaf Length}) + (12.63 * \text{Leaf Width}) + (1.96 * (\text{Leaf Length} - 7.51) * (\text{Leaf Width} - 3.64))$$

At each population, *P. quinquefolius* plants were selected using a stratified-random method: From 20 randomly selected clusters at each population, one plant per cluster was randomly selected from the dataset, given that the plant was present in 2012. However, if the population had fewer than 20 clusters, at least one plant was selected randomly from each cluster, and additional plants were randomly selected from previously used clusters to maintain comparable sample size among populations. Plants

that were not independent of each other (within two, five, or 10 meters, depending on the analysis) were removed from the dataset to avoid pseudoreplication (Sokal and Rohlf, 2012). However, any indicator species present in the microsite of these plants were marked as also present in the population. If the randomly selected plant did not emerge in 2013, or if the plant was browsed, the plant in closest proximity to the selected plant was used instead. For two unusually large, spatially expansive populations, additional individuals were sampled to increase sample size.

2.3. Putative Indicator Species

The putative indicator species used for this research were selected from a literature review (Anderson et al., 1993; Burkhart, 2013; Davis and Persons, 2014; Fountain, 1986; Roberts and Richardson, 1981), initial surveys of the overstory at each site, as well as unpublished opinions of an individual with extensive planting experience (Beyfuss, personal communication, 2013). Additionally, these species were selected based on ease of identification, which is important in indicator research, as this allows scientists, land-managers, and the general public to understand and use this research (Coote et al., 2013; Schiller et al., 2001). The indicator species list included herbaceous plants, shrubs, and trees (Table 1).

To determine if indicator species were able to predict *P. quinquefolius* performance, a modified ‘*Panax*-centric’ approach was used (Wixted and McGraw, 2009). Wixted and McGraw (2009) used this methodology to study the impact of invasive herbs, shrubs, and trees on *P. quinquefolius* plants. A research team identified the indicator species in the vicinity the *P. quinquefolius* plant at three different proximity scales for herbaceous, shrub, and tree species. Understory indicator herbaceous species

were counted as present if the putative indicators were found within two meters of the selected plant. Indicator shrubs were counted as present if the shrub was within five meters of the selected plant, and tree species were counted as present if the tree was within 10 meters of the selected plant (Table 1).

2.4. Data Analysis

For each year, any plants that were identified as new seedlings were removed from the analysis for that year only, due to atypical growth rates associated with the first year. Plants included in analysis had to be present for at least three years between the years of 2004 to 2013, to ensure that there were sufficient data points for a regression. The number of *P. quinquefolius* plants that met the criteria for independence, at the scale for herbs (2 m), shrubs (5 m), and trees (10 m), were as follows: N=367 for herbs, N=310 for shrubs, and N=236 for trees. Based on equations of Hunt (1990) and McGraw and Garbutt (1990), we calculated relative growth rate (RGR_{LA}) from 2004 to 2013 by finding the slope of the regression of natural log of leaf area on year for each plant.

To determine if there was a difference in *P. quinquefolius* RGR_{LA} among populations where indicator species were present or absent (population level analysis), a nested one-way ANOVA was used. Indicator species presence or absence at the population was the main effect, and population was nested within indicator species presence or absence class. Restricted maximum likelihood was used to estimate the variance components (SAS JMP, 2013).

Additionally, we asked if the presence or absence of a single indicator species affected *P. quinquefolius* performance, depending on the presence or absence of another indicator species. By selecting a combination of species of interest at the population

level, based on the other analyzes, we completed an “and/or” analysis to investigate if two indicator species were better at predicting plant performance, rather than an individual indicator. This analysis used a two-way nested ANOVA with the terms: Species 1 (levels: present/absent), Species 2 (present/absent), Species 1 X Species 2, and the nested term Population (Species 1 x Species 2).

For populations in which an indicator species was present, we asked if plant growth was greater in the microsite of the indicator, as compared to *P. quinquefolius* plants that were not within the microsite—at the two, five, and 10 meter scale (for herbs, shrubs, and trees, respectively). A two-way ANOVA was used for only the subset of populations having an indicator species of interest at the population level, but also having microsites within the same population that both had and did not have the indicator species of interest (Table 1). The two factors were population and indicator species (present/absent). We used the same dependent variable that was used in the population level analysis; relative growth rate. For all analyses in this study, if the residuals were not normally distributed, data were log transformed. Data were analyzed using SAS JMP Pro 11 (SAS JMP, 2013).

3. RESULTS

In the 26 populations studied, only one population did not have *A. saccharum* present, and similarly, only one did not have *A. triphyllum* present (Table 1). These were therefore excluded from population-level analyses. The next most common species present were *Polystichum acrostichoides* (Michx.) Schott and *Liriodendron tulipifera* L., at 22 and 20 populations, respectively. Due to limited replication, population level analysis of these two species may not be the most accurate.

3.1. Population Level Analysis

At the population level, for 14 of the 18 species examined, there was no difference in growth of *P. quinquefolius* when the putative indicator was absent vs. present ($p>0.05$). Of the indicator species that were able to successfully predict *P. quinquefolius* performance, a majority of these could be considered ‘contra-indicators,’ as their presence at a population resulted in lower growth rates. Growth in populations where *Aralia nudicaulis* L. was absent was 5.16-fold higher than growth in the populations having *A. nudicaulis* (Fig. 2a; $F=11.12$, $p=0.0067$). At populations that had *A. nudicaulis* present, the RGR_{LA} was no different from zero using a two-tailed t-test ($t=0.66$, $p=0.5135$), i.e., the plant was neither growing nor shrinking in size. Populations of *P. quinquefolius* had lower RGR_{LA} when *Acer rubrum* L. was present (Fig. 2b; $F=19.57$, $p=0.0007$). If *A. rubrum* was absent from a population, *P. quinquefolius* grew 11.48-fold faster relative to sites where this species was present. Populations with *A. rubrum* present had a mean RGR_{LA} that was not different from zero using a two-tailed t-test ($t=0.15$, $p=0.8849$). At populations with *Betula lenta* L. absent, *P. quinquefolius* had higher RGR_{LA} than *P. quinquefolius* that had *B. lenta* present at the population (Fig. 2c; $F=8.81$, $p=0.0055$). However, the RGR_{LA} of *P. quinquefolius* at populations with *B. lenta* was greater than zero using a two-tailed t-test ($t=2.57$, $p=0.0115$). There was a trend that there was an increase in performance if *Lindera benzoin* L. Blume was absent from the population (Fig. 2d; $F=2.98$, $p=0.092$), but the mean RGR_{LA} was greater than zero using a two-tailed t-test when the shrub was present at the population ($t=5.51$, $p<0.0001$).

Effects of multiple species on *P. quinquefolius* growth were not always additive. For example, there was a trend that the effect of *B. lenta* presence on the growth rate of

P. quinquefolius depended on the presence or absence of *Podophyllum peltatum* L. (Fig. 3a; $F_{\text{species 1} \times \text{species 2}}=3.86$, $p=0.0688$). In particular, *P. quinquefolius* tended to have a lower RGR_{LA} if a population had *B. lenta* present and *P. peltatum* absent. The RGR_{LA} of this combination was not different from zero using a two-tailed t-test ($t=-0.75$, $p=0.4547$), whereas all of the other means were greater than zero (*P. peltatum* present, *B. lenta* present: $t=4.38$, $p<0.0001$; *P. peltatum* present, *B. lenta* absent: $t=7.66$, $p<0.0001$; *P. peltatum* absent, *B. lenta* absent: $t=4.696$, $p<0.0001$).

There was also a trend for the effect of *B. virginianum* presence on the growth rate to depend on the presence or absence of *Tilia americana* L. (Fig. 3b; $F_{\text{species 1} \times \text{species 2}}=4.01$, $p=0.0529$). If the population had only one of the species present, there was a tendency for there to be a higher *P. quinquefolius* growth rate, but if both, or neither, species were present, there was reduced RGR_{LA} . All of the means differed from zero using a two-tailed t-test (*T. americana* present, *B. virginianum* present: $t=3.26$, $p=0.0015$; *T. americana* present, *B. virginianum* absent: $t=5.96$, $p<0.0001$; *T. americana* absent, *B. virginianum* present: $t=4.53$, $p<0.0001$; *T. americana* absent, *B. virginianum* absent: $t=3.53$, $p=0.0011$).

3.2. Microsite Level Analysis

In total there were 19 putative indicator species were included in the microsite analysis, as *Juglans nigra* L. was omitted from the study due to lack of replication within sites. If there was a high density of a single species within the site, where every plant was within a microsite of the indicator of interest, we excluded that population from the analysis, resulting in a smaller sample size. There was a lack of differences in the growth effects of 18 out of 19 indicator species examined at the microsite level. *L. tulipifera* was

a positive indicator for *P. quinquefolius* growth. If a *P. quinquefolius* plant was within 10 m of *L. tulipifera*, RGR_{LA} was 43.8% higher than for plants that were not near *L. tulipifera* (Fig. 4; $F_{\text{presence}}=6.60$, $p=0.0114$). The effect of *L. tulipifera* proximity on *P. quinquefolius* growth did not vary among populations ($F_{\text{pop} \times \text{presence}}=1.44$, $p=0.1701$).

4. DISCUSSION

4.1. Population Level

Two of the species, *A. saccharum* and *A. triphyllum*, were common among populations, and because of this, they were unable to be included in any population level analysis. The presences of *A. saccharum* and *A. triphyllum* have been observed in other indicator studies, and these species are often associated with *P. quinquefolius* habitat (Anderson et al., 1993; Burkhart, 2013). Both of these species have a large range, and are rather common throughout eastern North America (Bierzychudek, 1982; Lovett and Mitchell, 2004). The niche of these species could overlap the total range of *P. quinquefolius*, but perhaps still encompassing poor sites as well as good sites (Fig. 1b). An experimental study is needed to determine if these two species can predict performance at the population level, or if their broad niches encompass the niche of this species entirely.

At the population level, the presence of four indicator species (*A. nudicaulis*, *A. rubrum*, *B. lenta*, and *L. benzoin*) were able to predict reduced performance. These species are contra-indicator of high quality sites, even if their range extends into where *P. quinquefolius* might be found. One of these contra-indicators, *A. nudicaulis* was present at only three of the 26 populations. This understory herb can be found in mesic or dry-mesic forests (Roberts and Gilliam, 1995). Whitman et al. (1998) found that older ramets

of *A. nudicaulis* can survive on well-shaded sites with drier soils, whereas, *P. quinquefolius* grows best in moist, but well-drained soils (Li, 1995). As *A. nudicaulis* was only found at three sites, and can survive in drier conditions, the niche of *A. nudicaulis* may overlap a small section of the niche of *P. quinquefolius*, and not the area of the niche that represents maximized growth.

Another contra-indicator species whose niche likely does not mirror the niche of *P. quinquefolius* is *A. rubrum*. As reviewed in Burns and Honkala (1990), *A. rubrum* is a ‘super-generalist’ with a dense canopy, and the niche of *A. rubrum* may be one of the broadest of any native tree in North America. This hardy tree can grow on moisture extremes, from xeric to mesic soils, in a variety of elevations, pH levels, and soil textures. Wagner and McGraw (2013) determined that lower exposure to sunflecks is beneficial for the emergence of *P. quinquefolius* seedlings, but decreases the performance of adult plants. Therefore, the dense canopy of *A. rubrum*, and the propensity of this species to grow in soil moisture extremes, may be detrimental for the growth of this herb.

B. lenta was another tree contra-indicator. This species prefers well-drained, moist soils, but can grow in rockier and shallower soils (as reviewed in Burns and Honkala, 1990). As this species is associated with recently disturbed sites (as reviewed in Nowacki and Abrams, 1991), and is considered an intermediate successional species (Marks, 1975), the habitat is likely to be ill-suited for *P. quinquefolius* for several reasons. The canopies of a mature forest often have minor breaks in the canopy, allowing for scattered, yet predictable, patterns of sunflecks (Wagner and McGraw, 2013). The canopy structure of a secondary forest may not mirror the proper light requirements for

maximized growth of the study species. Sites that have *B. lenta* present may represent neither an ideal soil nor light environment for *P. quinquefolius*.

In addition to tree species, we identified *L. benzoin*, as a shrub with a tendency to be a contra-indicator. When *L. benzoin* is present at a site, the shrub is often a dominant species; only 23% of sites with *L. benzoin* present could be included in the microsite analysis due to the density of the shrubs at the population. Practical guides and experts often recommend planting/transplanting seeds or roots under or near *L. benzoin*, because that is often where *P. quinquefolius* can be found (Carroll and Apsley, 2004; Davis and Persons, 2014). However, *P. quinquefolius* may be growing under *L. benzoin*, not because the habitat supports optimum performance, but because birds, e.g., *H. mustelina* dispersed the seeds in this location (Hruska et al., 2014). Wood thrush (*H. mustelina*) is a primary disperser of *P. quinquefolius* seeds (Hruska et al., 2014). As this species tends to take shelter in shrubs (Vega Rivera et al., 1998), it could drop the seeds underneath the branches. Additionally, *P. quinquefolius* may be found under the dense, low branches of *L. benzoin*, because the branches may prevent harvesters from seeing the plant, or these branches may discourage browse from whitetail deer (*O. virginianus*). Browse can be detrimental to *P. quinquefolius* performance (McGraw and Furedi, 2005). *L. benzoin* has a dense canopy; while this cover may be beneficial for seedling germination, fewer sunflecks may reduce mature plant performance (Wagner and McGraw, 2013). While the growth rate was low, yet greater than zero, the benefits of *P. quinquefolius* being under *L. benzoin* (low deer browse and less likely to be found by harvesters) could outweigh the low growth problem when considering it as a planting spot.

344 While we identified four single-species contra-indicators, we also identified
345 combinations of species absence and presence that could predict *P. quinquefolius*
346 performance. At sites that had *B. lenta* present and *P. peltatum* absent, there was a trend
347 of reduced performance. Notably, *P. peltatum* grows best in moist soils, and often in
348 shady forest environments (Krochmal et al., 1974). *Betula lenta* prefers moist, well-
349 drained soils (as reviewed in Burns and Honkala, 1990), but has been found in xeric
350 environments (Nowacki and Abrams, 1991), as well as recently disturbed sites (as
351 reviewed in Nowacki and Abrams, 1991). The sites that have *B. lenta* present and *P.*
352 *peltatum* absent could be more xeric, which could explain the reduced performance.
353 Further, these forests may be younger. As *P. peltatum* comes into the understory slowly
354 after succession, since seed dispersal is often limited in clonal plants (Eriksson, 1993),
355 sites that have *B. lenta*, but are missing *P. peltatum*, may be too young to support
356 maximized herbaceous species performance.

357 Our research indicated that *P. quinquefolius* exhibited higher levels of growth in a
358 population with either *B. virginianum* or *T. americana*, but not both. In the eastern
359 deciduous forest, *B. virginianum* is often considered a positive indicator species for *P.*
360 *quinquefolius* (Anderson et al., 1993; Burkhart, 2013). Both *T. americana* and *B.*
361 *virginianum* are associated with high calcium soils (Burkhart, 2013; Burns and Honkala,
362 1990; Greer et al., 1997), as is *P. quinquefolius* (Burkhart, 2013). We posit that this
363 result could reflect chance, and indeed, statistically the 2-way interaction was only a
364 'trend'.

365 *4.2. Microsite Level Analysis:*

While we had several population level contra-indicators, we had only one indicator at the microsite level. *L. tulipifera* has a large range, and grows in moist, well-drained soils (as reviewed in Burns and Honkala, 1990; McCarthy, 1933); these soil conditions may be ideal for the growth of *P. quinquefolius* (Li, 1995). The crown of *L. tulipifera* is of medium density (McCarthy, 1933), which may provide a light environment that is suitable for mature plant growth (Wagner and McGraw, 2013). Additionally, *L. tulipifera* is a species that is associated with high levels of organic matter, and deep topsoil (as reviewed in Burns and Honkala, 1990; Elliott et al., 1999). These conditions associated with *L. tulipifera* suggest a nutrient rich environment that may be beneficial for *P. quinquefolius*. Our results should not be interpreted to mean that any site with *L. tulipifera* trees would represent quality habitat for reintroducing this rare plant. Since all the forested sites used in this study were >50 years old, the *L. tulipifera* trees growing in this forest were typically large. We are unable to conclude that sites with *L. tulipifera* are always beneficial (i.e. sites that are early successional forests of dense, young saplings); rather, we can conclude that sites with mature *L. tulipifera* may be beneficial for *P. quinquefolius* performance. With the other putative indicator species, some lack of significance of our microsite analysis may represent Type II error, as our small sample sizes could have resulted in incorrectly accepting the null hypothesis by chance.

4.3. General

The goal of this study was to critically evaluate putative indicator species for high-quality *P. quinquefolius* habitat across its range, in order to guide *in situ* conservation. As extension agents and hobby growers alike use indicator species for

reintroduction of *P. quinquefolius*, the quality of the indicator species used needs to be tested (Jørgensen et al., 2013). Surprisingly, only one of the putative indicator species had a positive relationship to *P. quinquefolius* performance, while the rest predicted poorer performance. A limitation to all indicator research with this species, so far, is that it does not account for if indicator species are found in similar abundances at sites without *P. quinquefolius* present. However, a strength of our study is that, while we followed this traditional methodology, we quantified the performance of *P. quinquefolius* at these sites. By incorporating growth and the presence and absence of indicators, we were able to evaluate the quality of the indicator species used.

Reintroduction projects can fail when the reintroduced species is planted in an ill-suited habitat (Godefroid et al., 2011). There can also be failure in agroforestry of *P. quinquefolius* (Davis and Persons, 2014). By understanding what species might be effective indicators of high quality habitat, we can reduce the waste of resources, time, and effort associated with unsuccessful reintroduction projects. How should we use indicator species if they predict negative growth of *P. quinquefolius*? The presence of contra-indicators at a site may suggest that reintroduction may not be as successful as reintroductions that occur at sites without contra-indicators.

A caveat to our research is that the climate is changing (Parmesan, 2006; Souther and McGraw, 2011), and now an important aspect of *in situ* conservation and forest management could be assisted migration (Millar et al., 2007). Some of the putative indicator species may have once represented an ideal habitat, but the current climate conditions could generate incompatibility between indicators and *P. quinquefolius*.

Extrapolating from our results, there should be a hierarchical process to evaluate site quality for *P. quinquefolius* reintroduction. First, the forest should be mature with a mostly closed canopy, mid-layer, and understory. The soil should be moist, but not wet, and there should be adequate, but not too dense shade (Wagner and McGraw (2013). Finally, when planting roots or seeds, sites with contra-indicators should be avoided (*A. nudicaulis*, *A. rubrum*, *B. lenta*, or *L. benzoin*), while planting *P. quinquefolius* close to large *L. tulipifera*. This may help in producing a successful reintroduction project to counteract the loss of *P. quinquefolius* populations, especially in an environment that is shifting.

In addition to using indicator species as a consideration of site quality when reintroducing this rare species, there are other important factors that can help increase the success of a reintroduction project; (1) Due to widespread poaching (McGraw et al., 2010), *P. quinquefolius* should be planted in areas that are isolated and generally protected from trespassers (Davis and Persons, 2014); (2) Sites should be chosen that are protected from future anthropogenic disturbances (Guerrant and Kaye, 2007), such as timbering (Chandler and McGraw, 2015), or surface mining; (3) As *P. quinquefolius* is susceptible to browse (McGraw and Furedi, 2005), sites should be selected that are protected from deer.

5. CONCLUSION

As changes to the environment are occurring, reintroduction of species to maintain or increase biodiversity may help species from becoming extinct (Bontrager et al., 2014). *P. quinquefolius* populations continue to decline from illegal harvesting (McGraw et al., 2010), deer browse (McGraw and Furedi, 2005), climate change

(Souther and McGraw, 2011), and land-use change (McGraw et al., 2013).
Reintroduction is a valuable, albeit high-stakes, method to return biodiversity to habitats
(Maunder, 1992). By understanding which indicator species are the best predictors of
suitable habitats for rare and valuable plants, land managers may be able to optimize
reintroduction practices. Reintroductions of rare species need to occur in areas of high
likelihood of success to ensure that species, such as *P. quinquefolius*, exist for future
generations.

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Table 1: List of indicator species along with number of populations in which they were found, as well as percent of those populations included in microsite analysis.

	Indicator Species	Common Name	Number of populations with indicator species present	Percent of those populations that were included in microsite analysis
Herbaceous	<i>A. pedatum</i>	northern maidenhair fern	8	38%
	<i>A. nudicaulis</i>	wild sarsaparilla	3	67%
	<i>A. triphyllum</i>	jack-in-the-pulpit	25	40%
	<i>A. canadense</i>	Canada wild ginger	9	22%
	<i>B. virginianum</i>	rattlesnake fern	15	73%
	<i>C. thalictroides</i>	blue cohosh	8	75%
	<i>P. peltatum</i>	mayapple	18	72%
	<i>P. acrostichoides</i>	christmas fern	22	59%
	<i>Actaea</i> sp.	black cohosh or doll's eyes	19	58%
Shrub	<i>H. virginiana</i>	American witch-hazel	4	50%
	<i>L. benzoin</i>	northern spicebush	13	23%
Tree	<i>A. rubrum</i>	red maple	4	75%
	<i>A. saccharum</i>	sugar maple	25	20%
	<i>B. lenta</i>	black birch	10	40%
	<i>F. grandifolia</i>	American beech	14	21%
	<i>J. nigra</i>	eastern black walnut	5	0%
	<i>L. tulipifera</i>	tulip poplar	20	55%
	<i>P. serotina</i>	black cherry	14	64%
	<i>Q. rubra</i>	northern red oak	16	63%
	<i>T. americana</i>	American basswood	16	31%

Figure 1: Graphical scenarios of the relationship between the niche of *P. quinquefolius* and several key 'putative indicator' species niches.

Figure 2: Effect of putative indicator species presence in the population on relative growth rate (RGR_{LA}) of *P. quinquefolius* plants over a 10-year period.

Figure 3: Effect of the presence or absence of two putative indicator species on relative growth rate (RGR_{LA}) of *P. quinquefolius* plants over a 10-year period.

Figure 4: The effect of the putative indicator species *L. tulipifera* presence in the microsite of *P. quinquefolius* on relative growth rate (RGR_{LA}) of *P. quinquefolius* plants over a 10-year period.

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Figure 1

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Figure 2

Figure 3

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Figure 4

Graphical Abstract:

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