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Testing lichen transplant methods for conservation applications in the southern Appalachian Mountains, North Carolina, U.S.A.

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Abstract. Three experiments were conducted to test new and established methods for lichen transplantation in the southern Appalachian Mountains. First, small fragments of *Graphis sterlingiana*, *Hypotrachyna virginica* and *Lepraria lanata* were placed on medical gauze attached to the most common substrate of each species to test the feasibility of transplanting narrowly endemic species with this established method. The medical gauze did not withstand the weather conditions at the transplant site, so a second experiment was conducted to test more resilient materials. Burlap, cheesecloth, medical gauze and a plastic air filter were directly compared for their use as artificial transplant substrates with *Lepraria finkii* as the test lichen. Third, transplants of *Usnea angulata* were established to test its amenability to transplantation by hanging fragments on monofilament. The first two experiments were established on Roan Mountain, North Carolina, and the third experiment at Highlands Biological Station, North Carolina. In the first two experiments medical gauze did not withstand local weather conditions and nearly all gauze fell from the trees within 6 months. The plastic air filter and burlap performed best as artificial substrates for transplants, with a 60% and 80% success rate, respectively. Cheesecloth remained attached to the trees, but only 20% of lichen fragments remained attached to the substrate after one year. In the third experiment *U. angulata* grew 3.5 ± 1.4 cm in the first five months and 1.8 ± 1.5 cm in the next four months, exceeding previously reported growth rates for this species. These results advance methods for conservation-focused lichen transplants, and expand established methods to a new region and new species.

Keywords. Translocation, assisted migration, restoration.

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There is now broad scientific consensus that anthropogenic change to the environment increasingly results in greater extinction risk for species worldwide (Butchart 2010; Ceballos et al. 2015; Dirzo & Raven 2003; IUCN 2016). While *in situ* conservation, preserving species where they already occur, is the ideal approach, fragmentation and degradation of the natural landscape force consideration of diverse actions (Chivian & Bernstein 2008; De Baan 2013). In the face of these threats translocations, i.e., movement of species to establish, re-establish, or bolster populations, are increasingly considered and conducted as a means to improve the conservation status of threatened and endangered species (Brichieri-Colombi & Moehrenschlager 2016; Guerrant 2013; IUCN/SSC 2013). North American animals illustrate the rapid increase in conservation translocations, with only one study published on the topic in 1974 compared to 84 such studies in 2013, and a total of 279 species over the 39-year period (Brichieri-Colombi & Moehrenschlager 2016). The ethics of species translocations have been thoroughly discussed. Major concerns include the potential for failure, negative impacts to source populations, impacts to the area where the species is introduced, and the resources required to successfully translocate a single species (Brichieri-Colombi & Moehrenschlager 2016; IUCN/SSC 2013; McLachlan et al. 2007). The most complete guide to address challenges and issues with conservation translocations was published by the International Union for the Conserva-

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tion of Nature (IUCN/SSC 2013). Regardless of the issues surrounding the practice, conservation translocations are likely to become increasingly important for all groups of organisms as resource extraction, loss of diverse ecosystems, and climate change progress in the coming century (Gallagher et al. 2015).

Lichen translocations, more commonly referred to as transplants, have been successfully carried out for numerous species. These studies have investigated diverse questions about lichen biology, including the impacts of air pollution on lichen physiology and survival (Brodo 1961; Ferry & Coppins 1979; Galun & Ronen 1988; Piccotto et al. 2011), morphology and development (Honegger 1996), and growth rates and seasonality (Muir et al. 1997). Numerous examples of lichen translocation specifically for conservation purposes also exist (Smith 2015). Some have been notable for their large-scale approach, with transplants involving over 1,000 individuals (Hazell & Gustafsson 1999; Sillett & McCune 1998). Others have been notable for their small scale, specifically focusing on transplanting thallus fragments and asexual reproductive propagules (Hilmo & Ott 2002; Kon & Ohmura 2014; Scheidegger 1995). The vast majority of the conservation studies have, however, focused on transplanting foliose, epiphytic species, especially from the genus *Lobaria*, while very few studies of crustose lichen transplants have been published (Smith 2015).

There are three main groups of transplant methods, most of which were designed and implemented for foliose and fruticose species. The simplest approach is to attach thalli or propagules directly to the substrate using an adhesive or staples (Honegger 1996; Lidén 2009; Scheidegger et al. 1995). There have been reports of negative impacts to the lichens where they are directly in contact with synthetic adhesives (Gilbert 1977). To avoid this, one study used only water as the adhesive for attaching lichen propagules and thallus fragments (Sillett et al. 2000). Another strategy to avoid negative impacts of adhesives is to move the underlying substrate along with the thallus, and directly attach the original substrate to a similar substrate at the new location. This has been accomplished by moving bark fragments or whole branches (Hilmo 2002), or taking circular portions of bark out of trees and inserting them into a hole of the same size on the target tree (Brodo 1961). The third method involves attaching the lichen to an artificial substrate that is then attached to, or hung from, the target substrate. The two methods used most frequently in this category are attaching sterile medical gauze to trees (Scheidegger 1995), and hanging lichens from monofilament (Denison 1988; McCune et al. 1996). The advantage of this method is that fragments of any size can be used. However, these methods do involve placing plastic and other synthetic materials into natural systems, and do not necessarily lead to the attachment of the transplanted lichens to the target substrate.

Few studies have investigated transplantation of crustose lichens, most of which sought to test hypotheses about lichen growth and physiology (Brodo 1968; Hale 1954, 1959; Seaward 1976). This is likely due to the challenging nature of crustose lichen transplants, and is evidenced by the mixed success of the few published studies. Two species, *Lecanora muralis* and *Opegrapha lythirga*, that grew on man-made substrates were successfully translocated and survived for at least three years and one year of monitoring, respectively (Seaward 1976; Smith 2015). *Dibaeis baeomyces* thalli that were moved on intact soil plugs from Newfoundland to Connecticut disappeared over the course of a nine year monitoring study (Hale 1954, 1959). The technical difficulty of transplanting crustose species, rather than limitations of their physiology, is often a hindrance to their establishment, precluding studies of growth (Brodo 1968). Transplanting crustose species is clearly one area of lichen translocation research that warrants further effort and attention to test and establish methods.

The southern Appalachians are a well-known lichen diversity hotspot, including many rare and narrowly endemic lichen species (Lendemer et al. 2013). The high elevations of this region host a unique assemblage of organisms, including an endemic fir, *Abies fraseri*, the spruce-fir moss spider, *Microhexura monitvaga* (Harper 1948; Manos & Meireles 2015; Seaborn & Catley 2016), and many lichens (Allen & Lendemer 2016). This area has historically been impacted by logging, acid rain and fog, and sweeps of invasive pests (Jetton et al. 2008; Milgroom & Cortesi 2004; White et al. 2012). Now it faces the potential of warming and shifts in seasonality with climate change (Ingram et al. 2013). Because this ecosystem hosts such an incredible diversity of species, including numerous endemics, and is at the same time facing significant threats, it is an important place to test and develop transplant methods. These methods will then likely be useful in other regions with similarly wet and humid climates.

In this study I used established protocols and tested new materials as artificial substrates to transplant five lichens species, including three crustose, one foliose, and one fruticose species, in the southern Appalachian Mountains of eastern North America. I transplanted three species that are narrowly endemic to the region, *Graphis sterlingiana*, *Lepraria lanata*, and *Hypotrachyna virginica* to test the suitability of medical gauze as an artificial substrate in the study area (Allen & Lendemer 2016). Then, I tested the utility of three other artificial substrates (burlap, cheesecloth, and a plastic air filter) for transplants using the common and widespread species *Lepraria finkii* (Lendemer 2013). One final species, *Usnea angulata*, was transplanted to test if established methods using monofilament loops and silicon sealant will work in the southern Appalachians (McCune et al. 1996). *Usnea angulata*'s distribution has declined substantially since the 1930's in eastern North America (Lendemer unpubl. data), and thus warrants further conservation attention.

METHODS

Sterile gauze transplants of narrow endemics. In the first experiment 2 × 2 inch (5.08 × 5.08 cm) pieces of sterile medical gauze were used as an artificial substrate and inoculated with fragments of *Graphis sterlingiana* (**Fig. 1A**) with underlying substrate, and *Hypotrachyna virginica*, and *Lepraria lanata* without underlying substrate. Portions of one or two thalli of each species were collected as source material to ensure that none of the source populations or individuals were negatively impacted by over-collecting leading to significant decline is populations. *Graphis sterlingiana* was collected from the Pisgah National Forest, near the intersection of US 215 and the Blue Ridge Parkway, Haywood County, North Carolina. *Hypotrachyna virginica* was collected from the Pisgah National Forest in the Black Mountains on Potato Hill, Yancey County, North Carolina. *Lepraria lanata* was collected from the Pisgah National Forest on Roan Mountain, Mitchell County, North Carolina.

Transplants of *Graphis sterlingiana*, *Hypotrachyna virginica*, and *Lepraria lanata* were established on Roan Mountain, North Carolina in May 2015. Three locations were installed for each species on different slopes, aspects, and altitudes. For *Graphis sterlingiana* and *Hypotrachyna virginica* at each site, one piece of sterile medical gauze was stapled at chest height and one was stapled two inches above the initial piece on the north, east, south, and west side of trees following the methods described by Scheidegger (1995). The thalli were cut into pieces using a razor blade and the small fragments were securely placed on the medical gauze using forceps. On each side of the tree a small fragment of source material was secured to the gauze, and the other piece was left blank as a control to test for independent lichen establishment and growth. The treatment and control pieces alternated in the upper and lower position. *Graphis sterlingiana* transplants were established on *Betula alleghaniensis* and *Hypotrachyna virginica* transplants were established on *Abies fraseri* or *Picea rubens*. *Lepraria lanata* transplants were established in a similar fashion, but gauze was attached to sheltered boulders and rock outcrops using silicon waterproof sealant. Thirty-six transplants of *Hypotrachyna virginica* and *Graphis sterlingiana*, and nine transplants of *Lepraria lanata* were established, for a total of 81 transplants and an equal number of controls. Photographs of each transplant were taken using a Nikon D3100 camera with Nikkor 105 mm macro lens. Transplant monitoring was conducted in November 2015, May 2016, November 2016, and March 2017. Monitoring consisted of photographing any remaining transplants and controls, along with scanning the material for living lichen fragments using a hand lens and noting their presence or absence.

Testing alternative transplant substrates with Lepraria finkii. The medical gauze failed as an artificial substrate, so I set up a second transplant study in November 2015 to directly compare the quality of a variety of materials for establishing crustose lichen transplants (**Figs. 1B & C**). The materials compared were 3 × 3 inch triangular pieces of burlap (measured at widest and longest points), 2 × 2 inch pieces of cheesecloth, 2 × 2 inch sterile medical gauze, and 2 × 2 inch pieces of Honeywell Filter A HRF-AP1 Universal Carbon Air Purifier Replacement Pre-Filter (referred to as 'air filter' throughout text). The three biodegradable substrates allow the possibility of the lichen to grow onto the underlying tree while the artificial substrates break

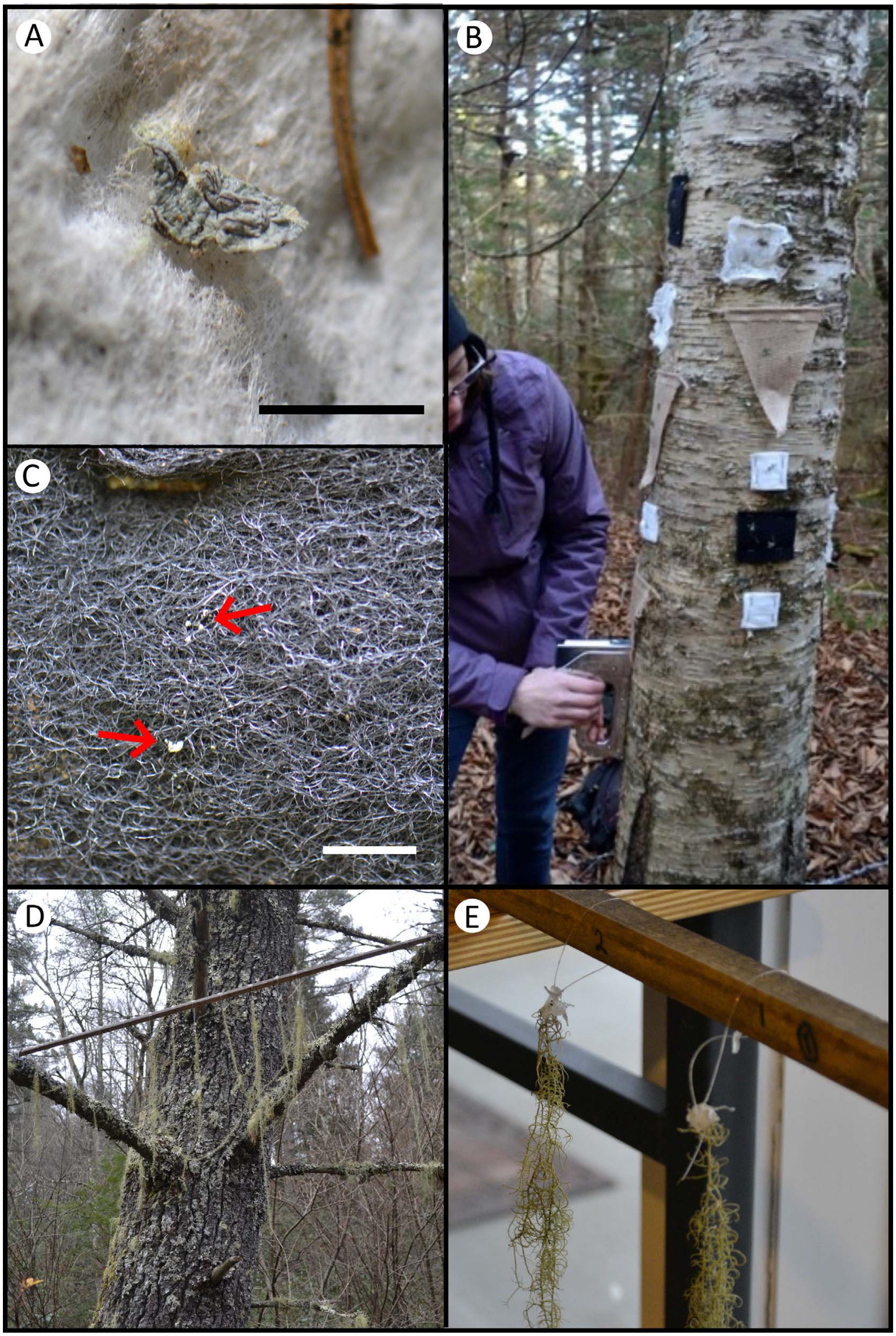

Figure 1. Diversity of substrates and species used in transplant studies. **A.** *Graphis sterlingiana* on sterile medical gauze (scale bar = 0.5 cm). **B.** Experimental set-up comparing four different artificial substrates. **C.** *Lepraria finkii* granules on plastic air filter, arrows point to granules (scale bar = 0.5 cm). **D.** *Usnea angulata* hanging on *Tsuga caroliniana* branches. **E.** Attachment of *U. angulata* to monofilament with silicon sealant.

down. The plastic air filter was included in this study because it was unclear whether or not any of the biodegradable substrates would persist long enough for the lichen to grow and establish on the underlying tree. For this portion of the study the common species *Lepraria finkii* was used, and source material was collected from Roan Mountain, North Carolina. The site was established near Carver's Gap on Roan Mountain. On a single *Betula alleghaniensis* one piece of each type of material was stapled to the north, east, south, and west side of the tree in a vertical line. Below the vertical line of treatment materials one extra piece of one of the substrate types was added as a control, with each side having a different material as a control. Small fragments of *L. finkii* were attached to each of the treatment patches on each side of the tree using forceps to transfer granules from the collected thallus to the substrate. On a second tree each of the four materials were stapled to the north side of the tree in a vertical line, along with an extra patch of medical gauze as a control. Again, *L. finkii* fragments were attached to each of these materials. Photographs of each transplant were taken using a Nikon D3100 camera with Nikkor 105 mm macro lens. Monitoring was conducted in May 2016, November 2016, and March 2017. Monitoring consisted of photographing any remaining transplants and controls, along with scanning the material for living lichen fragments using a hand lens and noting their presence or absence.

Monofilment transplants of Usnea angulata. On July 7 2016, one large thallus of *Usnea angulata* was collected from the ground where it had recently fallen from the tree along Turtle Pond Road on the south shore of the Cullasaja River in Macon County, North Carolina. The next day the thallus was cut into pieces between 19.5–63.5 cm long and attached at one end to monofilament loops with silicon sealant (**Fig. 1E**). The silicon sealant was allowed to dry for 1.5 hours before the loops were hung on a wooden dowel and secured at even spacing using silicon sealant to attach the monofilament to the wood. Each individual was numbered from 1–16 using Sharpie marker to write directly on the dowel. The initial length of each lichen fragment was measured as the distance between the silicon attachment point and the end of the axis furthest from the attachment point. The dowel was then hung on the Highland Biological Station campus between two branches of a large *Tsuga caroliniana* at the edge of a pond (**Fig. 1D**), roughly simulating the natural microhabitat of the species. In November 2016 and April 2017 the lengths of the transplants were remeasured as before.

RESULTS

The outcomes of the three transplant studies varied. The three southern Appalachian endemics established on sterile medical gauze had a 98.8% failure rate due to gauze detaching from trees. After the first experiment failed, the results of the second experiment showed that burlap and plastic air filters performed better than cheese cloth and medical gauze. The final experiment tested the viability of growing *Usnea angulata* transplants on monofilament. *Usnea angulata* grew rapid growth over the first five-month period, and grew more slowly over the following four months. Detailed results of each experiment are provided below.

Sterile gauze transplants of narrow endemics. Small fragments of *Graphis sterlingiana*, *Hypotrachyna virginica*, and *Lepraria lanata* were used to inoculate pieces of medical gauze in May 2015. When the transplants were monitored six months later in November 2015, only 21% of the gauze pieces remained attached to the trees and only 8.6% of the remaining treatments still retained lichen fragments. In May 2016, 12.3% of the gauze pieces remained, and 8.6% of these still hosted lichen fragments. In November 2016 and March 2017, 4.3% of the gauze pieces remained and only 0.6% (one piece of gauze) still retained a fragment of *Graphis sterlingiana* (**Fig. 1A**).

Testing alternative transplant substrates with Lepraria finkii. In November 2015, a second transplant study was established to compare plastic air filter, burlap, cheesecloth, and medical gauze as artificial substrates using *Lepraria finkii* as the inoculum. During the first monitoring point in May 2016 only one of the trees hosting transplants could be found for monitoring due to dense fog conditions and the presence of a bear. In November 2016 both trees were located and monitored, and all of the observations from the May monitoring were the same except for one cheese cloth transplant that lost its lichen fragment between May and November (**Fig. 2**). The six established pieces of air filter were all present, and there were still observable lichen fragments on all five of the replicates. The burlap

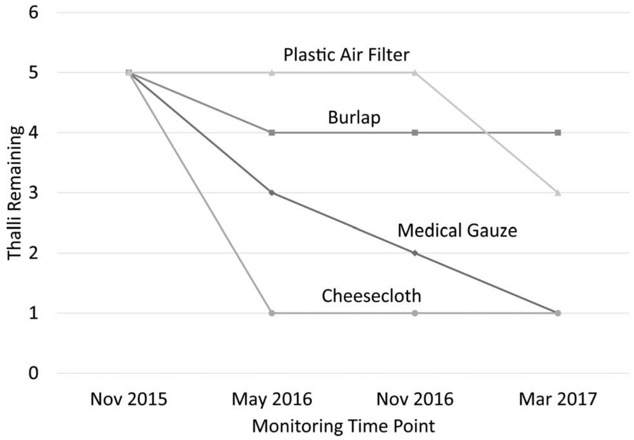

Figure 2. Number of remaining artificial substrates with lichen fragments attached at each monitoring time point for transplants of *Lepraria finkii* on different artificial substrates.

performed second best, with all six pieces of fabric remaining after one year, and four the five replicates still retaining visible granules. The cheesecloth was resilient, with all six pieces of material remaining, but the lichen did not stick well to the fabric and after one year only one piece of material still retained observable granules. The medical gauze performed poorly. Only two of the six piece of medical gauze remained attached to trees after one year, and neither pieces had observable lichen granules attached. Final monitoring was conducted in March 2017. At that time only one pieces of medical gauze remained attached the tree. The pieces of cheesecloth were still attached, but only one had fragments of *L. finkii*. Lichen fragments were absent from two of the five pieces of plastic air filter, leaving only three pieces (60%) with lichen fragments. The burlap performed best, with four of five pieces (80%) retaining lichen fragments. No measurable growth occurred in the duration of this study. Furthermore, the lichens have not yet grow onto the bark, though the burlap has begun to show signs of weathering.

Monofilment transplants of Usnea angulata. The *Usnea angulata* fragments averaged 33.3 ± 14.1 SD [19.5–63.5] cm long when first established. After five months they averaged 34.3 ± 12.4 SD [22–56] cm long. After an additional four months fragments averaged 34.9 ± 14.6 SD [16–60] cm long. The maximum length decreased because two of the thalli were shorter than when they were originally established and their ends were blunt, while the growing ends of the other transplants tapered to a point, suggesting that the two either broke or were eaten by animals. Broken thalli are excluded from the remaining results. In the first five months the transplants grew 3.5 ± 1.4 cm longer on average, gaining 1–6 cm in length. This represented a 5.6–17.7% length increase with an average of 11.7%. In the following four months the transplants grew an average of 1.8 ± 1.4 SD cm longer, growing 0–4.5 cm, or an average of 6% longer. In November 2016 fourteen of sixteen thalli displayed noticeable blackening to the outer cortex, potentially indicating that the thalli were damaged. During subsequent monitoring in March 2017 the blackening had disappeared.

Discussion

Established and new methods to transplant lichens were tested with mixed success for five species representing the three major lichen growth forms. In the first experiment gauze was used as an artificial substrate to transplant rare species. This method failed because the gauze did not hold up well to the weather conditions on Roan Mountain, which are characterized by near 100% humidity year-round and almost daily precipitation (Martin et al. 2015; Ulrey et al. 2016). Only one *Graphis sterlingiana* transplant remains intact on a protected face of a *Betula alleghaniensis* (**Fig. 1A**). Previous studies that used gauze as a substrate for lichenized propagules were successful (Kon & Ohmura 2014; Scheidegger 1995), so the success of medical gauze as a substrate likely depends on the environmental conditions at the transplant location and perhaps the quality of the gauze available.

After the failure of the first experiment, I conducted another experiment to directly compare other potential transplant materials and found that burlap performed the best (**Fig. 1B**). If the goal of the project is for the lichen to eventually grow onto the underlying substrate and establish itself while the artificial substrate degrades, then burlap is an excellent material. The large gaps between strands of material in the burlap leave sufficient space for the lichen to grow through and onto the underlying substrate while the burlap slowly degrades. This could provide a viable method for transplanting crustose lichens, especially those that produce lichenized asexual propagules. Furthermore, in dry habitats the burlap will hold moisture, providing a humid microhabitat for the transplanted fragments. The few previous studies reporting successful crustose lichen transplants were grown on artificial substrates (Seaward 1976; Smith 2015). This study supports the use of artificial substrates for crustose lichen transplants, and highlights the importance of preliminary tests of methods in regions where they have not been used in the past.

Successful conservation translocations of *Usnea angulata* are likely possible. The species grew quickly when attached to monofilament loops, averaging 3.5 and 1.8 cm of growth over a five and four month period, respectively. These results suggest a strong seasonal difference in the growth rate (Muir et al. 1997), and a faster growth rate than where the species occurs in Argentina (2.35 cm/year; Rodriguez et al. 2011). Now, longer monitoring and experimental attachment to diverse tree species are needed to fully assess the translocation potential for this species. The need for these experiments are especially urgent because *U. angulata* predominantly grows on dead or dying hemlocks (*Tsuga caroliniana*). As the trees continues to decline due to the invasive hemlock wooly adelgid (Jetton et al. 2008), it is unclear whether or not *U. angulata* will naturally transfer to a different substrate. Translocating this species from fallen, dead hemlocks to protected areas onto other tree species is one conservation action that can be taken to address its ongoing decline in eastern North America. As a general rule, care should be taken not to transplant invasive pests along with lichens to areas where the pest does not occur. This was not a concern in this project because the hemlock wooly adelgid causes nearly 100% mortality in hemlocks and has already impacted the study area (Jetton et al. 2008).

Here I presented three studies on lichen transplant methods with the aim of testing and expanding methods to a new region and new species. The technical difficulty of transplanting lichens is likely the largest challenge to implementation; a pilot study is recommended in most cases to first demonstrate success of the protocol in that region with common species of the same form as the transplant subjects. A number of other questions and considerations must be addressed by lichen researchers regarding translocation and reintroduction studies (Brichieri-Colombi & Moehrenschlager 2016; Guerrant 2013). First, we must consider what constitutes success. Survival and growth of the transplanted material are the most obvious, but growth of the species onto the underlying substrate, development of asexual and sexual reproductive structures, and recruitment (i.e., the production of viable offspring) would also be reasonable measures (Guerrant 2013). Second, the time frame of monitoring required to determine if a transplant is truly successful needs to be discussed. The time frame will vary by species and regions, but in many cases consistent monitoring for over 10 years and up to 30 is likely required to assess the average growth rate and life span of many lichens (Gilbert 1991, 2002). Finally, we must investigate what factors are the most important to ensure success of a transplant study. There are the technical aspects associated with the transplant methods, but other variables that must be considered include the depth of knowledge about the biology and ecology of transplanted species (Antoine & McCune 2004; Benedict 1990), size of the transplanted thallus fragments (Coxson & Stevenson 2007; Gauslaa et al. 2009), and the protection status of the site to which lichens are moved. A full assessment of factors that lead successful transplants is reliant on reports of failures, as well as successes. Continued focus on lichen transplant methods is essential as the need to reinforce, reintroduce, and translocate species for conservation increases.

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